VOLUME 64 • OCTOBER 1957 • NUMBER 4

PATHOLOGY

A Periodical Devoted to General and Experimental Pathology

AMERICAN MEDICAL ASSOCIATION PUBLICATION

TABLE OF CONTENTS

VOLUME 64 OCTOBER 1957 NUMBER 4

ORIGINAL ARTICLES

REGULAR DEPARTMENTS

A. M. A.
ARCHIVES of PATHOLOGY

Also the Official Organ of the AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

VOLUME 64 OCTOBER 1957 NUMBER 4

SUBSCRIPTION RATES

Price per annum in advance, including postage: Domestic, $10.00. Canadian, $10.50. Foreign, $11.50. Price to students, interns, and residents, $6.00 in U. S. & possessions.

Single copies of this and previous calendar year, $1.00 each. Back issues older than two years are available through Walter J. Johnson, Inc., 111 Fifth Avenue, New York 3. Future reprints of back issues will be available through Johnson Reprint Corporation, 111 Fifth Avenue, New York 3.

Checks, money orders, and drafts should be made payable to the American Medical Association, 535 North Dearborn Street, Chicago 10.

AMERICAN MEDICAL ASSOCIATION Publication

Published monthly by the AMERICAN MEDICAL ASSOCIATION. Editorial and Circulation Offices: 535 North Dearborn Street, Chicago 10, Illinois. Publication Office: Thompson Lane, Box 539, Nashville 1, Tennessee. Change of Address: Notice to the circulation office should state whether or not change is permanent and should include the old address. Six weeks' notice is required to effect a change of address. Second-class mail privileges authorized at Nashville, Tenn., Aug. 6, 1956.

Instructions to Contributors

Articles, book reviews, and other materials for publication should be addressed to the Chief Editor. Articles are accepted for publication on condition that they are contributed solely to this journal.

An original typescript of an article, with one carbon copy, should be provided; it must be double or triple spaced on one side of a standard size page, with at least a 1-inch margin at each edge. Another carbon copy should be retained by the author.

The main title of an article may not contain more than eighty characters and spaces; a subtitle may be of any length.

The author's name should be accompanied by the highest earned academic or medical degree which he holds. If academic connections are given for one author of an article, such connections must be given for all other authors of the article who have such connections.

If it is necessary to publish a recognizable photograph of a person, the author should notify the publisher that permission to publish has been obtained from the subject himself if an adult, or from the parents or guardian if a child. An illustration that has been published in another publication should be accompanied by a statement that permission for reproduction has been obtained from the author and the original publisher.

Oversized original illustrations should be photographed and a print on glossy paper submitted. Prints of a bluish tinge should be avoided. Large photomicrograph prints will be reduced in scale unless portions to be cropped are indicated by the author. The author should submit duplicate prints of roentgenograms and photomicrographs with the essential parts that are to be emphasized circled, as a guide to the photoengraver.

Charts and drawings should be in black ink on hard, white paper. Lettering should be large enough, uniform, and sharp enough to permit necessary reduction. Glossy prints of x-rays are requested. Paper clips should not be used on prints, since their mark shows in reproduction, as does writing on the back of prints with hard lead pencil or stiff pen. Labels should be prepared and pasted to the back of each illustration showing its number, the author's name, and an abbreviated title of the article, and plainly indicating the top. Charts and illustrations must have descriptive legends, grouped on a separate sheet. Tables must have captions. ILLUSTRATIONS SHOULD BE UNMOUNTED.

References to the literature should be limited to those used by the author in preparation of the article. They should be typed on a special page at the end of the manuscript. The citation should include, in the order given, name of author, title of article (with subtitle), name of periodical, with volume, page, month—day of month if weekly or biweekly—and year. References to books must contain, in the order given, name of author, title of book, city of publication, name of publisher, and year of publication.

AMERICAN MEDICAL ASSOCIATION

535 North Dearborn Street **Chicago 10**

a new concept in diagnosis

now! more rapid alkaline phosphatase tests for differential diagnosis of:

HEPATOBILIARY DYSFUNCTION:
- common duct stones
- stricture of the common bile duct
- biliary stasis
- fibrosis of the sphincter of Oddi

OBSTRUCTIVE JAUNDICE

OSTEITIS DEFORMANS (Paget's disease)

CANCER OF BONE

CANCER METASTASIS to the liver

IMPENDING JAUNDICE induced by drugs such as:
- tranquilizers
- steroids
- arsenic

---

no instruments needed
laboratory aides can learn quickly
results in 30 minutes or less

PHOSPHATABS* (alkaline) *with* TESWELLS*

AN ORIGINAL DEVELOPMENT OF WARNER-CHILCOTT RESEARCH

*for office and laboratory **screening** of alkaline phosphatase levels in nonicteric and icteric patients*

TO USE:

1. Place one tablet (PHOSPHATABS) in the special test tube.
2. Add 4 drops of serum or plasma.
3. Crush tablet with wooden applicator stick.
4. Let stand for 12-30 minutes depending on room temperature (determine time from curve in PHOSPHATABS package insert).
5. Add one drop of PHOSPHATABS COLOR DEVELOPER.
6. Compare color with color chart.

PHOSPHATABS* are available in a kit containing enough reagent tablets, color developer and TESWELLS* (controlled diameter tubes) for 48 determinations . . . $15. Phosphatabs and Teswells are available from your nearest laboratory supply distributor.

*TRADEMARK

For more information write to

Laboratory Supply Division
WARNER-CHILCOTT
MORRIS PLAINS, N. J.

exacting standardized thromboplastin—with the calibrated "controlled drop" dropette

Dropette brings these new advantages:

With the calibrated "controlled drop" dropette, the same rigidly measured quantity is assured for each test.

1. Accurate as a pipette, easier to use.
2. Saves valuable laboratory time.
3. Reduces possibility of contamination.
4. More economical (fewer pipettes used, fewer are broken).

* REGD. T.M.

SOLU-PLASTIN®—a superior thromboplastin solution

- Use of SOLU-PLASTIN® eliminates the chief error-factor in prothrombin-time determinations—inconstancy of the thromboplastin solution.
- Exactingly standardized—each batch is standardized against both normal and dicumarolized human plasma.
- Economical—there is no waste; no more solution is withdrawn than is to be used.
- Test is quick and simple—only four short steps are required.

Supplied: Bottles of 10 ml. (100 determinations) packaged with Dropette, nichrome wire and an equal amount of 0.0125 molar calcium chloride solution.
Detailed directions for the use of SOLU-PLASTIN® are enclosed in each package.

Send for LARGE DIRECTION SHEETS, convenient guides for laboratory personnel, and for the specially prepared PROTHROMBIN DETERMINATION RECORD SHEETS.

Schieffelin & Co./Since 1794

Laboratory Products Division
28 Cooper Square, New York 3, N. Y.

A.M.A. ARCHIVES OF
PATHOLOGY

Pathological Changes in Congestive Heart Failure

A Review of Findings in Fifty Cases

OGLESBY PAUL, M.D.; GORDON F. VAWTER, M.D.; ALBERT W. SCHWEITZER, M.D., and GEORGE M. HASS, M.D., Chicago

During the past decade the many studies of congestive heart failure which have appeared have largely emphasized physiological abnormalities. In this interest in recording functional variations it appears that too little attention has been paid to morphological changes in body tissues other than the heart, and a correlative study of clinical and pathological findings in congestive heart failure should be important and rewarding for the clinician, the physiologist, and the pathologist.

Selection of Material

Over the past eight years a group at the Presbyterian-St. Luke's Hospital, Chicago, has held weekly conferences at which both the clinical and pathological features of a case of some type of cardiovascular disease are discussed. Each case was selected by the pathology department from the current available autopsy material for its interest in the field of cardiovascular disease. From this group 50 consecutive cases have been selected in which the clinical records indicated by history and physical examination that congestive heart failure had been present (chronically in most cases) for at least 48 hours prior to death. The customary clinical criteria were considered to be acceptable, including chiefly a history of unusual shortness of breath (present at rest in 80% of the cases), associated in most instances with swelling of the lower limbs and often with abdominal swelling and discomfort. Confirmatory physical findings were considered to be the presence of respiratory distress, cyanosis, distention and pulsation of the neck veins, cardiomegaly, a diastolic gallop rhythm, pulsus alternans, signs of pulmonary congestion, hepatomegaly, and peripheral edema. It happened that no cases of heart failure due solely to lesions involving the right side of the heart are represented in this series, which may account for the fact that unusual shortness of breath on effort was cited in the history of all patients and orthopnea was described in 42; physical findings indicative of pulmonary congestion were recorded in 39, and hepatomegaly was noted in 34.

Not only were the clinical records reviewed in detail, as well as the autopsy protocols, but also the histologic slides were thoroughly reviewed in each case and where necessary new sections were cut so that the material might be more completely studied.

Nine of the fifty cases were studied during life by one of us.

Certain of the findings which will be mentioned below do not bear a constant or casual relation to congestive heart failure itself but have other important implications for the whole field of cardiovascular disease and will be referred to at times because of their own interest.

Age.—The average age of the patients in this series was 57, the youngest being 15 years of age and the oldest, 84.

Sex.—Thirty-two of the patients were males, and eighteen were females.

Types of Heart Disease.—The types of heart disease encountered in this series are indicated in Table 1. It will be seen that the group is representative of those conditions most commonly encountered in practice and in a general hospital population. Unusual conditions are two cases of primary amyloidosis with cardiac involvement, one

Submitted for publication March 22, 1957.

From the Departments of Pathology and Medicine, Presbyterian-St. Luke's Hospital.

TABLE 1.—*Types of Heart Disease*

	No. Cases
Coronary heart disease	11*
Coronary and hypertensive heart disease	10
Rheumatic heart disease	10*
Rheumatic and hypertensive heart disease	4*
Rheumatic and coronary heart disease	3
Hypertensive heart disease	3
Coronary and hypertensive heart disease with calcific aortic stenosis	2*
Primary amyloidosis	2
Coronary heart disease and calcific aortic stenosis	1
Hypertensive heart disease with dissecting aneurysm	1
Hypertensive heart disease with calcific aortic stenosis	1
Idiopathic hypertrophy	1
Carcinomatosis involving pulmonary arteries and pericardium	1
	50

*One case in each group had bacterial endocarditis.

case of idiopathic hypertrophy, and one case with adenocarcinomatosis (? primary site) with hemopericardium and signs of progressive cardiac tamponade and failure of two weeks' duration. A diagnosis of hypertension was made when the majority of the blood pressure readings recorded during previous and the final hospital admissions were above 150 systolic and/or 95 diastolic. A diagnosis of coronary heart disease was made when there was marked diffuse atherosclerosis of the coronary system with or without infarction or where local atherosclerosis was associated with an infarct.

Duration of Heart Failure.—In 37 of the 50 patients review of the histories indicated that symptoms and signs of congestive heart failure had first appeared six months or longer prior to death, and in 25 of the 50 these evidences had been present for a year or more. In only four cases did it appear that congestive heart failure had been present for less than a week (and, as stated, no case was included in which cardiac failure had been manifest for less than 48 hours). It is obvious that this study is essentially a study of chronic congestive heart failure, although it should be stated that in many instances it was apparent that following the initial episode of cardiac failure adequate or tolerable compensation was regained and retained until a few days or weeks before death.

Heart Weights.—An indication of the severity of the cardiac disease present can be obtained from the finding that the average heart weight for the series was 544 gm., compared with a normal range of 250 to 400 gm. Twenty-seven of the hearts weighed 500 gm. or more (and seventeen of these weighed 600 gm. or more) and 13 weighed 400 to 500 gm. Ten of the hearts weighed less than 400 gm., the smallest being a 290 gm. heart in a female patient with rheumatic heart disease with mitral regurgitation and stenosis. The average heart weight for the males was 594 gm. and for the females, 455 gm.

Findings

Pericardial Effusion.—It has been stated [1] that one cause of a significant pericardial effusion may be cardiac failure itself. True, we have at times seen sizable pericardial effusions at operation on patients with acquired heart disease; most often, however, they are seen in those having active rheumatic fever as determined by microscopic study of atrial tissue. Our study of the 50 patients in this series shows, however, that in patients with chronic congestive heart failure a large pericardial effusion is rare. Only 8 patients of the 50 had an effusion greater than 50 cc. in amount (excluding the one case in which a hemopericardium existed due to neoplastic involvement). Of these eight, the effusion was small (100 cc. or less) in five, and the largest effusion was only 250 cc., being found in a patient with an active rheumatic myocarditis, which may well have been important in its production. These relatively small effusions were encountered in patients with varying types of heart disease. They were not found particularly in patients having the largest hearts or the longest history of congestive heart failure, but it did appear that a significant pleural effusion (200 cc. or more in one pleural cavity) was usually present when a significant pericardial effusion existed.

Weights of Certain Organs.—Before discussing body tissues other than the heart, it is of interest to note the average gross fresh weights of certain of the organs to be discussed, as compared with the normal. It is apparent that with the exception of the brain, an increase in organ weight is invariably found. Table 2 lists these weights.

TABLE 2.—*Weights of Organs*

Organ	Cases, No.	Average Weight, Gm.	Normal Range, Gm.
Brain	22	1,330	1,250–1,450
Lungs (both)	48	1,170	650– 800
Liver	49	1,663	1,400–1,650
Spleen*	44	221	120– 150
Kidneys (both)	48	351	250– 280
Adrenals (both)	42	18	12.6– 14.0
Pancreas	46	95	85– 90

*Omitting cases with bacterial endocarditis and amyloidosis.

Brain.—Gross and microscopic examination of the brain and brain stem was made in 26 of the 50 cases. The average brain weight (available on 22 cases) was 1330 gm., well within normal limits.

Symptoms of severe cerebral dysfunction (convulsions, hemiplegia, aphasia, coma, and prolonged disorientation) as far back as 15 months prior to death were described in the records of 8 of the 26 patients. In five of these eight the symptoms could be correlated with gross cerebral lesions with cerebral infarction or malacia due to embolism from a fibrillating left auricle in patients with rheumatic mitral disease. No satisfactory discrete gross lesions could be found to explain disorientation occurring in a 74-year-old man during the last two weeks of his life or in two other patients, aged 15 and 68, with rheumatic stenosis of the mitral and tricuspid valves in the former and stenosis of the mitral, tricuspid, and aortic valves in the latter, who had convulsive seizures 48 and 24 hours, respectively, prior to death.

Microscopic observations showed that possible evidences of cerebral edema were present in the majority of the sections in both the cerebral hemispheres and the basal ganglia. However, we do not wish to emphasize this observation, believing that in cerebral tissue it is difficult to interpret such histologic changes with complete satisfaction and to distinguish true edema from alterations attributable to early postmortem change and to fixation technique. Indeed, the presence of normal brain weights is good evidence against the presence of cerebral edema.

The brains of four patients also showed microscopic areas of hemorrhage in the cerebral hemispheres and basal ganglia (and in the cerebellum in one and meninges in two of these). These could be correlated with the underlying cardiovascular disease: one patient had severe hypertension and the other three had sources of embolism—in bacterial vegetations on the mitral and aortic valves and left auricular or ventricular thrombi. The patient with bacterial endocarditis had also intraventricular hemorrhage. No areas of hemorrhage could be specifically attributed to congestive failure per se.

The hazard to the brain of thrombus formation in the left side of the heart is further pointed out by the observation that of 11 cases with gross and/or microscopic areas of infarction or malacia of the brain and brain stem, 5 had known auricular fibrillation or flutter for at least two weeks prior to death and 2 had subacute bacterial endocarditis of the mitral and aortic valves. Three of the remaining four had myocardial infarction without demonstrable mural thrombosis in the heart chambers at autopsy, although such may have existed previously, and one had probable auricular fibrillation.

Thyroid and Parathyroid.—No specific lesions attributable to congestive failure were found in the thyroid or parathyroid glands.

There was little evidence of increased thyroid activity in the 49 cases in which sections of the thyroid were available for review, and indeed the glands appeared more atrophic or hypoplastic than hyperplastic. The follicular epithelium was chiefly flat in 19 cases, was cuboidal in 23, and was columnar in only 4. Moderate hyperplasia was seen in only 2, and mild hyperplasia was noted in 13, but in 3 of these the process was focal with a predominant diffuse atrophy.

Lungs.—The average weight of the two lungs together (data are available on 48 cases) was 1170 gm., with a range from 510 to 2700 gm., as compared with the normal range of 650 to 800 gm. Only four pairs of lungs (from four female patients) weighed less than 800 gm., and these were from patients with combined coronary and hypertensive heart disease. There were nine pairs of lungs from eight male patients and one female patient having weights greater than 1500 gm., and in this group were five patients with aortic or mitral valvular heart disease, as well as four patients with coro-

nary or hypertensive heart disease or both. The nine cases with the heaviest lungs also had very heavy hearts (average 647 gm., as compared with the average of 544 gm. for the whole series and 594 gm. for all the males) and extremely heavy livers (average 2246 gm., as compared with the average of 1693 gm. for the whole series and 1813 gm. for all the males). In seven of the nine symptoms of congestive failure had appeared six months or more before death.

The right lung, as is normal, was heavier than the left lung in 38 of the 48 cases, and the two lungs were equal in weight twice. In five of the remaining eight in which the left lung was heavier than the right either compression of the right lung by a large pleural effusion or handling of the left lung by the surgeon a few hours prior to death with increase in edema formation may have explained the findings.

It is interesting to observe that the average lung weight for the 31 males was 1321 gm., while that for the 17 females was 904 gm. This difference of over 400 gm. is greater than can be explained by the usual difference in lung weights for the two sexes and is likewise difficult to correlate with etiological types of cardiovascular disease or with the duration of heart failure.

Evidences of pneumonia as characterized by alveolar wall and space inflammation, chiefly peribronchial, were found in 9 of the 50 patients, varying in age from 34 to 74. As a group these patients did not have an average age greater than the average for the whole series, and no single type of heart disease was conspicuously represented. Likewise, there was no clear association with the duration of symptoms of cardiac failure.

Excluding mild degrees of change, fibrosis of the alveolar walls was noted in 21 of the 50 cases, (Fig. 1). This was considered to be Grade II (moderate) in 13, Grade III (marked) in 6, and Grade IV (extreme) in 2. The sections from the 13 cases showing Grade II fibrosis came from patients with a variety of types of heart

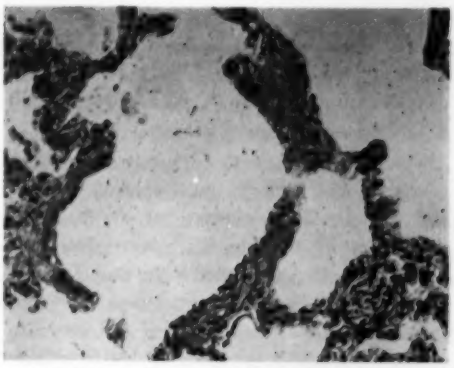


Fig. 1.—Section from the lung of a 51-year-old man who had been in cardiac failure for a period of seven years. Thickened alveolar walls are shown surrounding clear alveolar spaces.

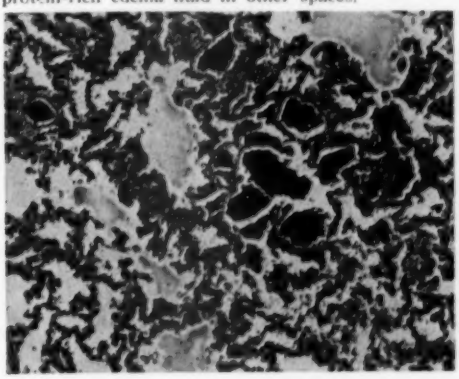
disease (seven had coronary or hypertensive heart disease or both) in whom there did not appear to be any unusual degree of emphysema, alveolar wall or space inflammation, or bronchial inflammation; these patients were not limited to the older age group, and the duration of symptoms of congestive heart failure was not unusually long. It is important to observe by contrast that of the eight patients with marked or extreme—Grade III and IV—alveolar-wall fibrosis there were six who had mitral stenosis and one with mitral regurgitation. Thus seven of the eight showing severe alveolar wall sclerosis were patients with mitral valve disease. This characteristic has of course been noted by others. Not all patients dying with mitral stenosis show extreme alveolar fibrosis. One patient dying with mitral stenosis and coronary heart disease with symptoms of heart failure for 6 months showed mild alveolar fibrosis, another with pure stenosis and symptoms for 8 years also showed only mild changes, and three with symptoms dating back 5 to 22 years had essentially no fibrosis. Some degree of fibrosis was encountered in 10 of the 13 patients with mitral stenosis, however, and in one patient with pure mitral regurgitation. These changes could not be correlated with the age of onset of heart failure.

As might be expected in a group of persons chiefly in middle and old age, emphysema was not uncommon, although there were no instances of emphysema of extreme degree. There were 12 patients with Grade II and 3 with Grade III emphysema. The average age of this group of 15 was 61, slightly above the average for the whole series.

It is interesting to observe that in the whole group of 13 patients with mitral stenosis there were 2, aged 45 and 68, with Grade III emphysema; 3, aged 51, 52, and 72, with Grade II emphysema, and 3, aged 33, 36, and 45, with Grade I emphysema. It is difficult to conclude from such limited material that heart disease or mitral valve lesions in particular were significant in the production of much emphysema. There was no evidence in the series as a whole that emphysema had resulted in the production of a chronic cor pulmonale.

One typical finding in the lungs of patients dying with congestive heart failure is the presence in the alveolar spaces of large macrophages laden with hemosiderin ("heart failure cells") (Fig. 2). Diffuse or focal aggregates of hemosiderin-laden macrophages may occasionally be detected by x-ray. Varying degrees of hemosiderosis were encountered in this series: 14 cases were classified as Grade I; 13, as Grade II; 7, as Grade III, and 4, as Grade IV—a total of 38 out of 50 cases. As might have been anticipated, hemosiderosis was found in 9 of the 10 cases with gross pulmonary infarction; it was Grade II or III in 7 of the 9. It was also seen in all of 14 patients who had hemoptysis regardless of cause and was graded from II to IV in 11 of these. There also appeared to be a definite relation to mitral stenosis. Three of the 4 patients with Grade IV hemosiderosis had mitral stenosis, and, conversely, 10 of the 13 patients with mitral stenosis had hemosiderosis of a degree ranging from Grade II to IV. Hemosiderosis was also encountered in all the other types of heart disease represented in the series, without, however, a direct relation to duration of symptoms of cardiac failure or with a predilection for specific types except as noted above in mitral stenosis.

Fig. 2.—Section from the lung of a 33-year-old man with rheumatic heart disease with mitral stenosis who had been in congestive heart failure for four years. Hemosiderosis is present, with clumps of hemosiderin-laden macrophages (appearing black) filling some alveolar spaces and with protein-rich edema fluid in other spaces.



The presence microscopically of large amounts of pink-staining homogeneous protein precipitate in the alveolar spaces was considered to be evidence of pulmonary edema. A total of 23 of the 50 cases showed this on review of the sections. There was a nice correlation of gross lung weights with degrees of pulmonary edema as classified microscopically. The pulmonary edema present was found in all types of heart disease without emphasis on any group, although it may be noted that neither patient with tricuspid stenosis showed this finding.

At least from these autopsy data, it was apparent that pulmonary vascular disease did not entirely "protect" the alveolar spaces from filling with edema fluid. Eleven of twenty-eight cases with Grade II to Grade IV pulmonary arteriolar sclerosis also showed Grade II to Grade IV pulmonary edema microscopically. The incidence of edema was less, however, with pulmonary capillary fibrosis; here, of 16 cases with pulmonary capillary fibrosis of Grade II degree or more, only 4 showed Grade II to IV pulmonary edema. The relationship

to alveolar wall fibrosis was also considered by using the same method of grading, and it was found that of the 21 patients whose lungs showed this finding (Grade II or more), there were 5 with pulmonary edema of Grade II or more severity.

In the mitral stenosis cases in particular, excluding those with associated organic tricuspid disease, there was no clear inverse relationship between the degree of pulmonary vascular disease and the degree of protein precipitate in the alveolar spaces. It cannot be stated, though, that more pulmonary edema might not have been found had not this vascular and alveolar wall disease existed.

There was no obvious relation of the extent of pulmonary edema to the duration of congestive heart failure, age of the patient, heart weight, liver size or pathology, or presence of pulmonary infarction.

Gross pulmonary infarction was encountered in 10 of the 50 cases and was rarely diagnosed correctly clinically. Seven of the ten had been in congestive heart failure for one year or more, the remaining three having had evidences of failure for one month, four and one-half months, and six months, respectively. A source for embolism was usually not demonstrated as routine dissection of the superficial and deep venous drainage of the lower limbs is not done. Auricular fibrillation had been present in 3 of the 10 cases. Hemoptysis had been present in only 2 of the 10, and none gave a "typical" history of sudden chest pain, collapse, or pleurisy.

The right lung was the site of the infarcts in six cases, both the right and the left lung were involved in three, and the left lung was the sole site of infarction only once. Not only was it striking therefore that the right lung was involved in 9 out of 10 cases, but it was of additional interest that in the 9 cases in which the exact lobes involved were described, the right lower lobe was involved in 7. Believing that this might have been just chance, another group of 25 patients not in this series who were listed in the pathological files as having gross pulmonary infarction due to pulmonary embolism were reviewed. In 13 only one lung was involved, with the right lung infarcted in 10 and the right lower lobe also involved in 9, and in 12 both lungs were the sites of infarction, with the right lower lobe a site of infarction in 11. In no instance in the 10 cases in our series or in this additional group of 25 were the right middle, right upper, or left upper lobes the sole site of infarction.

Pulmonary arteriosclerosis of moderate (Grade II) or marked (Grade III) degree was found in 14 of the 50 cases. In the total group of 14, there were 6 with mitral stenosis (45% of those with mitral stenosis in the whole series), 5 of whom were under the age of 60, and 4 with coronary disease, all of whom were 60 years of age or over, with two showing a moderate emphysema. Important in the genesis of this vascular change, aside from the relation to mitral disease, are the facts that in every case showing Grade II or III arteriosclerosis either evidences of congestive heart failure had been present for from one to seven years before death (10 cases) or, if present for less than one year, they were associated with moderate (Grade II) emphysema (4 cases).

Pulmonary atherosclerosis of Grade II degree was observed twice; of Grade III degree, three times, and of Grade IV degree, once. Of these six cases, one had amyloid deposits in the pulmonary arterial bed associated with an obliterative endarteritis, one was a 74-year-old man with coronary disease and moderate emphysema, and four had mitral stenosis (the oldest of these four patients being only 36 years of age).

Pulmonary arteriolar sclerosis of Grade II degree was seen in 21 cases; of Grade III degree, in 6, and of Grade IV degree, once—a total of 28 cases, excluding those with only minimal or Grade I disease (Fig. 3). There were 16 of the 28 with predominant arteriolar sclerosis and little or no arteriosclerosis. The cardiac diagnosis in 9

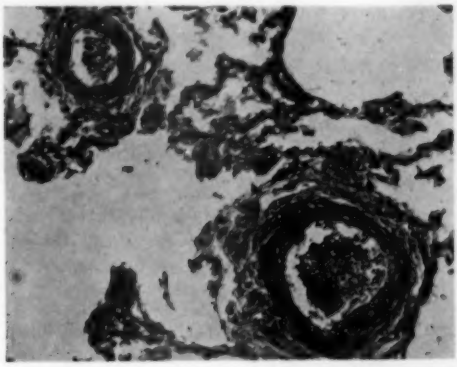


Fig. 3.—Section from the lung of a 58-year-old female diabetic patient with hypertensive heart disease who had been in congestive heart failure for 16 months. Well-marked arteriolar and small artery sclerosis are seen.

of these 28 was mitral stenosis (70% of the total group dying with mitral stenosis), and 7 had coronary heart disease. The only patient with Grade IV disease had mitral stenosis, and three of the six with Grade III changes had either mitral stenosis or regurgitation. Of the 17 patients not having mitral valvular disease, only 6 appeared to have had cardiac failure for less than six months, and of these, 5 showed Grade II or greater pulmonary alveolar fibrosis or emphysema.

Pulmonary capillary fibrosis of Grade II to IV severity was observed 16 times, 14 of which cases were also included in the group with pulmonary arteriolar sclerosis mentioned above. All 5 of the cases of Grade III or IV severity had either mitral stenosis or mitral regurgitation. Of the total group of sixteen, nine had a cardiac diagnosis of mitral stenosis and two had mitral regurgitation.

Dilated lymphatics of Grade II to IV severity were described in the lungs in 40 of the 50 cases. This was graded as II in 24, III in 13, and IV in 3 others. It was noted both centrally and peripherally in the lungs, although chiefly the latter, and appeared to be quite nonspecific as far as etiologic types of heart disease were concerned.

Pleural Effusion.—For the purposes of this study, a significant pleural effusion was considered to be present when 200 cc. or more of fluid were found in one pleural cavity. A significant effusion was encountered in 28 cases,* being bilateral in 20, involving only the right pleural cavity in 7, and involving only the left pleural cavity once. It is important to note that in this last case the right pleural cavity had been entirely obliterated and was therefore unavailable. Our experience thus confirms that of others [2] in stressing the rarity of an isolated left pleural effusion in congestive heart failure.

We were unable to find any positive correlation of the incidence of pleural effusion with the duration of heart failure. Furthermore, there was no predominance of any one type of heart disease. In correlating the presence of pleural effusion with alveolar wall or capillary or arteriolar fibrosis of Grade II or greater degree it was found that one-half of those in each group showed a significant effusion, and where all three lesions were present (12 cases), 7 had significant effusions. Correlation of the pathological and x-ray findings suggested that a pleural effusion may develop extremely rapidly.

Liver.—Liver weights were available in 49 of the 50 cases, and sections were prepared for microscopic study in all 50 cases. The average weight of the livers was 1693 gm.; the range in the group was from 950 to 2950 gm. The average liver weight in the females was 1485 gm., as compared with a normal average of 1400 gm., and in the males it was 1813 gm., as compared with a normal average of 1650 gm.

In 3 of the 50 cases the liver sections were considered to be within normal limits. It is of interest that although the patients had symptoms and signs of heart failure for from six months to four years the terminal episodes did not represent the end-stage of

* One case is not included which had a pleural effusion which may have been infected and not due to cardiac failure.

a steady and progressive downhill course but were instead sudden and unexpected. The fact that the liver sections were normal does not imply that the liver weights were not increased, however, as two of the livers weighed 2515 and 2200 gm., respectively.

In nine other patients liver sections showed only histologic changes consistent with mild (Grade I) acute central congestion. Acute central congestion was defined as dilatation and engorgement of the central veins and centrilobular hepatic sinusoids surrounding histologically normal cords of liver cells. Here again the liver weights showed in several cases a marked increase. Four of the nine livers weighed over 2000 gm. and the average for the three females was 1716 gm. (range, 1500 to 2050 gm.) and for the 6 males, 1977 gm. (range, 1600 to 2500 gm.). This was definitely above the average for the whole series and indeed above the average for any of the later groups to be discussed showing more marked histologic abnormalities. It is evident that the largest livers may show little histologic change, even lacking the usual evidence of sinusoid dilatation and engorgement. There was no satisfactory correlation of duration of heart failure or type of heart disease with these minimal visible cellular and vascular alterations, except as indicated for the three normal livers.

In the remaining 38 cases more striking abnormalities were obvious, and these may be divided into three main categories—congestion, central necrosis, and alteration of the hepatic vasculature, or "cardiac cirrhosis."

Degrees of congestion from Grade II to IV, with or without atrophy of the hepatic cells, were noted in 21 patients; since 3 of these were also associated with considerable alteration of the hepatic vasculature they will be considered later, thus leaving 18 cases, or 36%, of the whole series in which congestion with or without cellular atrophy was the chief finding.

Acute central congestion alone of Grade II or more intensity was observed in only 3 of the 18 cases. These patients had been in some congestive heart failure for from 5 days to 10 years, although their terminal episodes of acute failure in no case appeared to have extended over 10 days. The liver weights ranged from 1200 to 2275 gm.

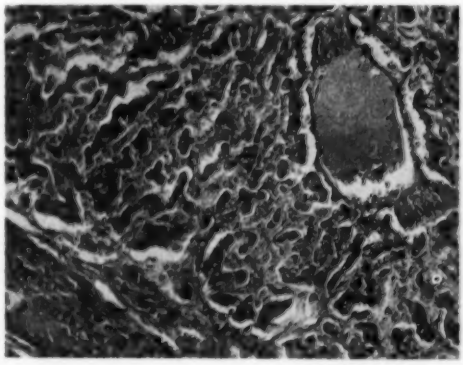
In the remaining 15, degrees of central congestion of Grade II to IV with atrophy were observed as shown by shrinkage and hyperchromatism in hepatic cells in centrilobular distribution, often with decrease in lobular size (Figs. 4 and 5). The average liver weight here was 1572 gm., with a range from 1090 to 2300 gm., and the duration of clinical heart failure varied from two weeks to four years, with 12 of the 15 giving a history of congestive failure of six months or longer.

Central necrosis, that is necrosis of centrilobular cells, of Grade II to IV, with or without inflammation, was observed in 12 cases, or 24% of the whole series.

TABLE 3.—*Classification of Hepatic Pathology*

	No. of Cases	Per Cent
Normal or mild central congestion	12	24
Acute central congestion, with atrophy	3 15	36
Central necrosis, with inflammation	7 5	24
Altered hepatic vasculature	8	16
	50	100

Fig. 4.—Section from the liver of a 52-year-old woman with hypertensive and coronary heart disease who experienced a myocardial infarct six days before death. A dilated central vein is shown (upper right) surrounded by atrophic liver cells.



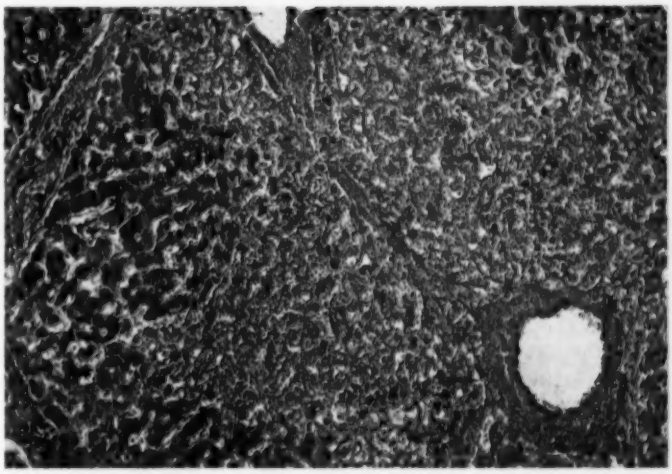


Fig. 5.—Section from the liver of a 34-year-old man with idiopathic hypertrophy of the heart who had been in cardiac failure for two years. Two dilated thickened hyalinized hepatic central veins are shown connected by strands of fibrous tissue. In focal areas of adjacent tissue is marked atrophy associated with extreme dilatation of sinusoids.

In seven of these, the central necrosis was not accompanied by significant degrees of inflammation (Fig. 6). It was associated with Grade III central congestion in one, Grade II central congestion with atrophy in four, and Grade III central congestion with atrophy in two. The average liver weight in this group was 1858 gm., with a range from 1260 to 2950 gm. Shock had been present for 12 hours prior to death in one and for 24 hours prior to death in another; in the remainder the blood pressure was maintained above shock levels until four hours or less before death. Four of these cases had associated pulmonary infarction.

The final group, of 8 cases, or 16% of the whole group, had what might best be termed altered hepatic vasculature, although many employ the term "cardiac cirrhosis." Histologic changes were those of central perivenous fibrosis and/or sclerosis of the central vein and intralobular veins, with an apparent or real increase in the prominence of small vascular channels ("translobular capillaries") between the portal areas and extending for various distances into the lobule toward or connecting with central veins (Fig. 7). These vessels sometimes

Fig. 6.—Section from the liver of a 29-year-old man with mitral stenosis and regurgitation and an active myocarditis (rheumatic). Acute central necrosis is present, a wide band of necrotic tissue surrounding a central vein with more normal liver cords in the periphery.

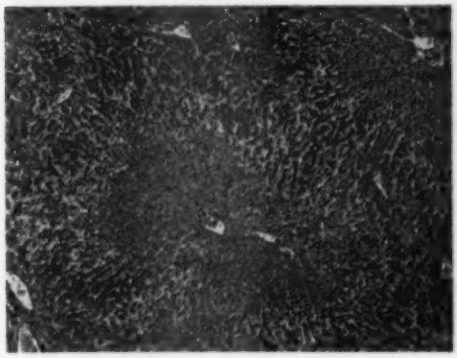
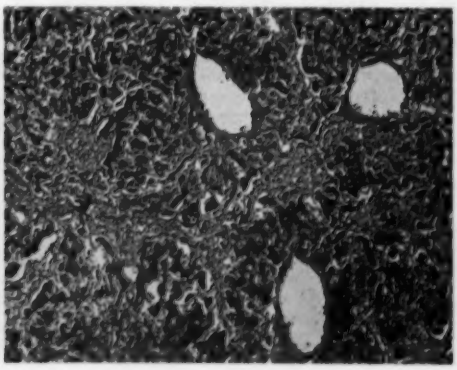


Fig. 7.—Section from the liver of a 33-year-old man with mitral stenosis and cardiac failure of eight years' duration. Gross distortion of hepatic architecture is seen. Three central veins are included, with interconnecting vascular channels, fibrosis, and disorderly groups of liver cells.



delimited irregular or slightly nodular groups of hepatic cells, possibly regenerative, which lent a scalloped border to the lobule. As might be anticipated, in these cases with chronic vascular changes there was less evidence of acute congestion than in the groups mentioned previously and there was little or no central necrosis of the type just described. Fibrosis around the central vein was prominent in only four of the eight cases, and we believe that the fundamental and significant lesion should be considered as vascular with changes in the stroma playing a secondary role. The average liver weight for the group was 1389 gm., with a range from 950 to 1800 gm. Four of these had coronary and/or hypertensive heart disease, and four had rheumatic heart disease, with mitral stenosis being present in all of the four (combined with aortic valve disease in two and with tricuspid stenosis in one).

Ascites.—Ascites in amounts of 250 cc. or more was present in eight cases, but since one of these instances probably was that of an infected fluid, only seven cases will be considered as being solely due to congestive heart failure. In no case could independent portal cirrhosis or carcinomatosis be held responsible, no portal vein thrombosis was demonstrated, and although serum protein levels were available in only four of the seven cases, those which were recorded were sufficiently within normal limits to exclude hypoproteinemia as an important factor. The total duration of heart failure in this group ranged from 1 month to 10 years, with six of the seven having had evidences of failure for 6 months or longer. In no instance was the final episode of acute failure shorter than one month. There appeared to be no relation of the presence of ascites to any one type of heart disease, patients with coronary, hypertensive, aortic valvular, and mitral and tricuspid valvular stenosis being included. None of the patients with ascites had splenic infarcts.

It is worthy of comment that in four of the seven a Grade I or II alteration of the hepatic vasculature was present. This represents one-half of the cases with altered hepatic vasculature.

The average heart weight in the group was 581 gm., approximately 40 gm. above the average for the whole series. The average spleen weight was 276 gm., 56 gm. above the average for the series, but the range was wide (140 to 525 gm.). In five of the seven a significant pleural effusion was also found.

Gastrointestinal Tract.—Changes in the esophagus, stomach, small intestine, and colon attributable to the presence of congestive heart failure were common. Histologic sections of the esophagus were available for review in 43 cases; of the stomach, in 45; of the small intestine, in 42, and of the colon, in 43. A gross description was available in 49. Table 4 indicates the frequency of venous dilatation, capillary engorgement, and edema of the intestine wall encountered on study of the histologic sections. Over half of the cases showed significant changes of this type in the stomach,

TABLE 4.—*Tabulation of Certain Histologic Changes in the Gastrointestinal Tract*

	Dilated Veins					Engorged Capillaries					Edema				
	Grade					Grade					Grade				
	I	II	III	IV	Total	I	II	III	IV	Total	I	II	III	IV	Total
Esophagus (43 cases)	9	4	4	1	18	3	5	3	1	12	2	7	4	3	16
Stomach (45 cases)	14	15	5	0	34	7	14	11	0	32	12	10	8	2	32
Small intestine (42 cases)	9	19	7	1	36	11	13	7	1	32	12	14	6	3	34
Colon (43 cases)	15	6	4	0	25	9	6	5	0	20	10	10	2	2	24

small intestine, and colon, and somewhat less than half demonstrated such changes in the esophagus.

An acute esophagitis was noted microscopically in six cases, and one of these showed ulceration. In none of these six patients had a Levin or other indwelling tube been passed, although such was the case in still another patient in whom marked gross ulceration was noted; this we believe may well have been due to irritation from the tube.

Chronic esophagitis was observed histologically 10 times.

An acute gastritis was found in only 12 patients, with a gross gastric ulcer in 1 and several superficial gastric ulcerations in another. A chronic gastritis was diagnosed in 11 others. Petechiae were observed three times. It was not possible to correlate these observations with any specific type of heart disease, duration of heart failure, presence of shock, or particular changes in the liver. An atrophic gastritis was also seen in 12 cases, ranging in age from 52 to 75.

When the small bowel was studied, acute or subacute duodenal ulcers were identified in five patients, one of which had perforated. (One of the five also had a gastric ulcer.) Shock had been present for 24 hours before death in two of the five and for 12 hours in a third. A hemorrhagic duodenal ulcer, possibly an infarct, was seen in a sixth patient, with coronary heart disease, aortic stenosis, and auricular fibrillation, who also had renal infarcts. A careful review of the records of these patients gave no indication of a past ulcer history. Of the five duodenal ulcers clearly not due to infarction from embolism, one occurred in a patient with primary amyloidosis and cardiac failure, three occurred in patients with coronary heart disease with acute as well as old infarction, and one occurred in a patient with coronary and hypertensive heart disease with an ancient myocardial infarct. It is possible that the ulcer in the patient with amyloidosis was secondary to amyloid deposits in the small vessels of the duodenum narrowing the blood supply and leading to ischemic necrosis; there was no evidence of amyloid deposits in the wall of the duodenum itself similar to that reported by Lindsay and Knorp[8] as giving rise to gastric ulceration. Omitting the ulcer possibly due to embolism with infarction and that seen in the patient with primary amyloidosis, this incidence of at least 8% active duodenal ulcers compares with an over-all incidence of 3.7% active duodenal ulcers (including those with perforation and hemorrhage) among 1085 routine adult autopsies performed at the Presbyterian Hospital, Chicago, during the four-year period 1948 through 1951.

An acute or chronic duodenitis, jejunitis, or ileitis was described in eight other cases.

Study of the colon was less revealing, a colitis being present in only three cases, one of which occurred in a patient who had severe diarrhea three days before death. In addition, a large rectal ulcer with marked necrosis of the bowel wall was found in a 68-year-old man with myocardial infarction who, while in shock, received eight aminophylline suppositories over the 36-hour period prior to death.

There was no correlation of any of these inflammatory gastrointestinal changes with amounts of mercurial diuretic administered.

Pancreas.—The pancreas was examined grossly and microscopically in all 50 cases, but weights were available in only 46. The average weight was 95 gm. (at the upper limit of normal), with a range of from 50 to 190 gm.

Congestion of the pancreas was found to be present in 32 of the 50 cases. Lymphedema was observed in 14 cases. These changes could not be closely related to the duration of cardiac failure or to any type of heart disease.

An interstitial pancreatitis of Grade I degree was also seen in 20 of the cases, with Grade II involvement in 8 cases. Among these latter 8 were 6 with a history of cardiac failure dating back 2 years or more (in the total group of 50 there were 20 with

such a history). There was no relation of the incidence of interstitial pancreatitis to the age of the patient or type of heart disease. Hyalinization of the islets of Langerhans, when present, appeared to be closely associated with the presence of diabetes mellitus.

Lobular atrophy was found in 22 cases. This finding appeared nonspecific, unrelated to type of heart disease or duration of cardiac failure, but possibly associated with the increasing age of the patients. Also difficult to relate specifically to congestive heart failure was the finding in some cases of abnormally large nuclei in the islet cells and a decrease in the presence of secretory granules in acinar cells.

Arteriolar sclerosis in the pancreas, as in other abdominal organs, bore a close relation to the presence of hypertension.

Spleen.—The spleens were examined grossly and microscopically in all 50 cases, and weights were available for all. The average weight was 226 gm. (as compared to a normal range of 150 to 200 gm.), but if the six cases with subacute bacterial endocarditis and primary amyloidosis are excluded the average weight was 221 gm., with a range from 60 to 525 gm. In this group of 44 there were 9 spleens weighing 300 or more gm., 12 weighing 200 to 300 gm., and 8 weighing 150 to 200 gm., the heaviest spleen (525 gm.) being found in a patient with hypertensive heart disease and with congestive failure for 16 months. Of the nine patients with splenic weights of 300 gm. or more, seven had had cardiac failure for six months or more. Only three of the spleens (weighing 280, 650, and 390 gm., respectively) were recorded as being palpable during life.

Dilatation of the sinusoids was commented on in 35 cases, excluding those with subacute bacterial endocarditis and primary amyloidosis. There was no correlation of hepatic pathology with these findings.

Fibrosis of the red pulp appeared to bear a close relation to the duration of congestive failure but not to histologic evidence of red pulp engorgement. Again excluding the cases with bacterial endocarditis and amyloidosis, there were 11 instances of Grade I fibrosis; 4, of Grade II fibrosis; 4, with Grade III change, and 1, with Grade IV fibrosis of the red pulp. Seven of the nine cases with Grade II to IV fibrosis had been in clinical cardiac failure for 2 years or more, and the other two, both showing only Grade II change, had shown evidences of failure for 9 and 18 months. Three of the nine had a significant (Grade II or more) degree of altered hepatic vasculature, and, similarly, three of the nine (including two of the three with an altered hepatic vasculature) showed ascites. There was no good correlation with the types of heart disease or with splenic weights.

Atrophy of the white pulp of some degree was a common finding and probably not more marked than would be expected in any group of cases falling in this age group. This was Grade I in 18, II in 9, and III in 7.

Splenic infarcts were observed in nine instances. One of these occurred in a patient with primary amyloidosis with thrombi in the left auricle; six occurred in patients with rheumatic heart disease and auricular fibrillation; one occurred in a patient with coronary and hypertensive heart disease and auricular fibrillation, and one was seen in a patient with coronary heart disease and multiple infarcts of the left ventricle who very likely had mural thrombus formation at some time, although such was not demonstrated at autopsy. Of 19 cases with auricular fibrillation or flutter, 7 thus showed splenic infarction.

Kidneys.—The kidneys were examined grossly and microscopically in 50 cases, although weights were on record in only 48. The average weight of the right kidney was 175 gm. and of the left kidney, 176 gm., with an average combined weight of 351 gm. (which compares with the normal range of 250 to 280 gm. for the combined weight). The range of individual kidney weights was

from 85 to 400 gm.; in 34 cases both kidneys weighed 140 gm. or more apiece.

Dilatation of the glomerular space was observed in 23 of the 50 cases. In 27 of the 50 a protein-rich fluid filled the glomerular spaces, a finding which was believed to be usually a terminal anoxic phase not necessarily related to previously observed albuminuria. A nonspecific "membranous" glomerulonephritis was described in three patients. Two others, both with rheumatic heart disease and auricular fibrillation, showed a focal glomerulonephritis, possibly embolic in origin.

An irregular hypertrophy of the epithelium lining the convoluted tubules was encountered in 11 of the 50 cases. There was no relation of this change to the history of and recorded use of mercurial diuretics, although it is possibly significant that these patients gave a long history of congestive heart failure (9 of the 11 had been in cardiac failure for two years or more). The exact relation of other types of therapy to this finding is difficult to evaluate, but at least two patients who had been on a very strict low-sodium diet for many months prior to death failed to demonstrate this change. Hydropic degeneration was also seen in 23 cases.

Actual necrosis of the epithelium of the convoluted tubules was observed in five instances. Here again there was no relation of this finding to the use of mercury and none to the presence of shock or duration of cardiac failure or presence of central necrosis of the liver. A true nephrosis was considered to be present in three of these, two of whom had received no mercurial diuretic at all as far as could be determined and one of whom had received none for the 10 months prior to death, although quite large amounts had been administered before this. No typical mercurial nephrosis was found in any case, although it was suspected in two others, both of whom were given mercurial diuretics during the last few days of life. It is pertinent to comment that 26 of the 50 patients had been known to have had parenteral mercurial therapy, with multiple injections having been given to at least 15 of these.

Dilatation of the convoluted tubules was remarked on in 27 of the 50 cases. Those having the most extensive and diffuse dilatation tended also to have the heaviest renal weights. A protein-rich fluid was noted in the tubular spaces in 35 of the 50, with "copper"-colored casts in 4.

Edema of the interstitial tissue was present in 22 of the 50. Only 1 of these showed a Grade III change, with 9 being classified as Grade II and 12, as Grade I. This finding did not bear any close relation to the gross kidney weights or to any morphologic type of heart disease.

Renal infarcts were encountered in 13 patients. Seven of these occurred in patients with rheumatic heart disease and mitral stenosis with auricular fibrillation; two, in patients with primary amyloidosis and thrombi in the left auricle; one, in a patient with coronary disease and aortic stenosis and auricular fibrillation; one, in a patient with coronary disease and mural thrombi in the left ventricle and auricular fibrillation, and 1, in the patient with adenocarcinomatosis and a hemopericardium. Thus, 12 of the 13 either had demonstrated sources of embolism in the left side of the heart or had an auricular arrhythmia known to predispose to thrombus formation in the left auricle with the hazard of peripheral embolism. It is particularly significant that they occurred in 6 of the 13 patients with mitral stenosis (all of whom had auricular fibrillation) and in both patients with primary amyloidosis.

Arteriolar sclerosis was noted in 27 of the 50, and, as expected, all of the patients with severe disease were hypertensive and the severity of the process was independent of age. Arteriosclerosis appeared related to both age and the presence of hypertension.

Vascular scars were diagnosed in 27, again occurring most often and most severely in those with hypertension.

Adrenal Glands.—Gross and microscopic examination of the adrenal glands was made in 49 cases, and the weights were recorded for 44. Omitting two cases with cortical adenomas, the average combined weight of the two adrenals was 18 gm. (as compared to a normal range of 12.6 to 14.0 gm.), the range being from 7 to 38 gm. Although at first glance it appeared that there was a direct relation between heart size and adrenal weights (the average heart weight for the 13 patients with adrenal weights of 20 gm. or more was 644 gm. and for the 11 patients with essentially normal weights [10 to 15 gm.] was 534 gm.), a scatter plot indicated a lack of adequate correlation for the whole group. Some relation may exist between the heaviest adrenals and the duration of congestive heart failure. Eight (61%) of the thirteen cases with combined adrenal weights of 20 gm. or more gave a history of cardiac failure of one or more years, whereas such a history could be obtained in but six (35%) of the seventeen cases with adrenal weights ranging from 15 to 20 gm. and five (45%) of the eleven cases with adrenal weights of 10 to 15 gm. More impressive was the fact that all four of the patients with bacterial endocarditis had adrenal weights exceeding 20 gm.; these four ranged from 23 to 38 gm., with an average of 29 gm. There was no other correlation with etiologic types of heart disease.

Hyperplasia of the adrenal cortex was determined to be present on microscopic section in 17 of the 49 cases. Two cases with cortical adenomas were encountered. There was no consistent relation between these microscopic findings and the total adrenal weight, heart weight, type of heart disease, or type of hepatic or pulmonary pathology. All of those showing Grade II or more cortical hyperplasia had been in cardiac failure for six months or longer. No advanced atrophy was encountered. The two patients with cortical adenomas were cases with rheumatic heart disease and mitral regurgitation and with hypertensive heart disease, respectively, with periods of cardiac failure of 1½ years and 16 months.

Acute passive congestion of the zona reticularis was described in 12 cases.

The adrenal medulla was the site of acute passive congestion in 36 cases. Chronic passive congestion of the medulla was also described in 18 (16 of which also showed acute congestion). Here again, the acute and chronic changes were marked (Grade III, eight cases) in patients with long histories of cardiac failure; only one patient gave a history of failure for as short a period as six months, and the remaining seven varied from one year to seven years. Moderate (Grade II) chronic changes also were seen only in patients with histories of congestive heart failure ranging from six months to seven years. Petechial hemorrhages of the adrenal medulla were mentioned six times (none of these had bacterial endocarditis), and frank hemorrhage occurred once in a 51-year-old patient with mitral stenosis.

Arteriolar sclerosis of the adrenal was again closely related to the presence of hypertension.

Finally, mention may be made of thickening of the adrenal capsule seen in 23 cases and extramedullary hematopoiesis in the capsule in 26 (a finding which could not be closely related to the duration of cardiac failure).

Bone Marrow.—The essential alterations in the bone marrow constituents in the 46 cases in which microscopic sections were available for review are indicated in Table 5.

Erythroid hyperplasia tended to be most notable in patients with long histories of

TABLE 5.—*Alterations in the Bone Marrow Constituents in Forty-Six Cases*

	Grades				
	I	II	III	IV	Total
Erythroid hyperplasia	17	8	3	0	28
Erythroid hypoplasia	4	1	0	0	5
Myeloid hyperplasia	9	4	0	0	13
Myeloid hypoplasia	6	0	0	0	6
Lymphoid hyperplasia	12	2	0	0	14
Lymphoid hypoplasia	2	1	0	0	3
Megakaryocytic hyperplasia	2	1	0	0	3
Megakaryocytic hypoplasia	2	1	0	0	3

cardiac failure but without relation to the age of the patient or specific type of heart disease or presence of pulmonary parenchymal or vascular disease. Among the 11 cases with Grade II or III erythroid hyperplasia there were 10 with histories of congestive heart failure dating back six months or more and 8 with a history dating back a year or more. Among those with only Grade I erythroid hyperplasia, no significant correlation with the duration of cardiac failure was obvious. Only a few cases showed erythroid hypoplasia—and only one showed even a moderate depression of the red cell elements—and no conclusions can be drawn regarding these.

Changes in the myeloid and lymphoid series were not as common as in the erythroid series. Myeloid hyperplasia, as found in 13 cases, was never marked and may have been related in 4 of these to bacterial endocarditis and pulmonary infarction. Only mild myeloid hypoplasia was evident in six cases, five of whom were over the age of 60. Similarly, hyperplasia of the megakaryocytic series was limited to three cases—all of whom showed multiple infarcts of two or more organs; and hypoplasia was also discovered three times (one of these showed a terminal pulmonary infarct).

Review of the bone marrow sections also disclosed sinusoid and venous engorgement in 20 cases.

Prostate.—The prostate was examined grossly and microscopic sections were available for review in 21 of the 38 males.

A chronic prostatitis was a common finding, being noted in 15 of the 21 cases. There was no correlation with the duration of cardiac failure.

Vascular engorgement of the prostate was likewise the rule and was found in 20 of the 21 prostates. Edema of the prostate was similarly common and was seen in 18 of the 21 cases. Both vascular engorgement and edema were unrelated to age, the severest degrees of these changes including relatively young males.

Testes.—Gross and microscopic examination of the testes was performed in 28 of the 32 males.

Depletion of the spermatogenic cells was observed in the majority (18) of the patients. This could only be considered as being related to cardiac failure in the younger age group, and it is therefore of some interest that a Grade III reduction in cells was found to be present in the 15-year-old boy with rheumatic heart disease, a Grade II reduction was present in a 42-year-old man, and a mild or Grade I depletion included patients aged 48, 45, and 29. Spermatogenesis was found to be reduced in 24 of the 28, and here also a Grade IV reduction included two patients, aged 45 and 15, a Grade III reduction included patients aged 54 and 42, a Grade II change was found with men aged 29 and 48, and a Grade I reduction included a 42-year-old man. A mild orchitis was observed in seven, with a moderate and a marked orchitis each being found once.

Edema and vascular engorgement of the testes were similarly common. Testicular edema existed in all but one of the 28 cases. Capillary dilatation was noted in 17, and venous dilatation, in 13.

Comment

The above recitation of gross and microscopic findings obviously includes items not due to congestive heart failure as well as a great many which are clearly the result of cardiac decompensation. It was thought best, however, to list as we have done all of the chief abnormalities, indicating each by numbers of cases and grades of severity so that the material is available for reference and correlation.

The findings we have recorded may fall into any one of three groups: (1) those which are actually of such slight deviation from normal gross anatomy and cellular morphology that they should be broadly considered as normal (it is with this in mind that we have tended to minimize Grade I changes); (2) those findings which are

common to any prolonged or severe wasting disease independent of the presence of cardiac failure; (3) those abnormalities which appear to be dependent on some type of congestive heart failure for their existence.

A completely satisfactory analysis of these three possibilities would require a study of two additional groups of cases—first, a series of 50 patients dying essentially instantaneously from localized trauma without opportunity for generalized tissue changes, and second, a group of 50 patients dying from a prolonged or severe illness with minimal if any cardiac weakness until the last few moments of life. The Presbyterian-St. Luke's Hospital does not have in its autopsy files material for such analyses. The first group would be best obtained from coroner's cases; in lieu of such material, we have relied upon the customary accepted standards of normality for our comparisons, depending upon the experience and wisdom of the many members of our pathology department who have given their time to this project. The second group would be difficult indeed to procure with certainty and might in any case be unimportant. For the end-picture of congestive heart failure must and does include metabolic, biochemical, and nutritional factors which, while not unique in cardiac failure, are of vital importance to the economy and survival of the person. It is with these comments in mind that the appraisal of our findings is made.

Regardless of the type of heart disease present there is an accumulation of protein-rich edema fluid in all the organs of the body studied except the bone marrow. This is least conspicuous in the brain and most obvious in a spongy organ like the lung, where its very presence threatens life itself. In other organs, such as the gastrointestinal tract, pancreas, spleen, adrenal, prostate, and testes, the functional significance of collections of this fluid is less immediately obvious, although its role in the prostate in producing an obstruction to the bladder neck is well known clinically.

Associated with this process is a generalized increase in organ weights. This may to some extent be offset in the course of time by secondary cellular atrophy and necrosis and with the production of variable increases in the amounts of connective tissue —as may occur in the liver, spleen, and lung. The actual tissue wasting which occurs with prolonged cardiac failure is again known clinically.

Further, with the changes in venous and pulmonary arterial and capillary pressures incident to cardiac failure, vascular wall alterations occur, some of which become characteristic of certain types of heart disease, as is seen in the pulmonary vascular disease associated with mitral stenosis and mitral regurgitation (but not unique for these conditions).

Another vital factor frequently producing an abrupt termination of the course of the disease is the high incidence of embolic phenomena in almost all organs, apparent from the statistics we have enumerated. These are common with rheumatic mitral disease with auricular fibrillation or flutter but are also found in other conditions with auricular arrhythmias, with bacterial endocarditis, with mural thrombi secondary to myocardial infarction or primary amyloidosis, and with thrombi in the pelvic and more important the leg veins. Under this category come 10 cases with pulmonary infarction, 9 cases with splenic infarction, 13 patients with renal infarction, 1 pancreatic infarct, and probably 11 instances of cerebral infarction.

From our data it is reasonable to conclude that a large pericardial effusion due solely to congestive heart failure is rare and that when a significant effusion is found it is usually in association with a pleural effusion.

Study of the 26 brains in the series would indicate that significant increases in brain weights do not take place, and there was no evidence to support a concept of cerebral edema. Symptoms of severe cerebral dysfunction were most often attributable to

embolism from the left auricle or left ventricle. The evidence of severe hepatic damage in many of the cases would raise the possibility that terminal rises in blood ammonium levels might be important in producing stupor or coma.

Analysis of the pulmonary findings was particularly interesting. Extensive increases in lung weights took place, correlating well with estimates of the degree of alveolar edema present. Those showing the greatest amount of alveolar wall fibrosis were patients with long-standing pulmonary hypertension due to mitral valve disease (and some degree of alveolar wall fibrosis was seen in 10 of the 13 patients with mitral stenosis). Emphysema was less striking in patients with mitral disease, only 5 of 13 with mitral stenosis showing Grade II and III changes. Pneumonia, chiefly peribronchial, was less often encountered than we had expected, being found in but 9 of 50 cases, and was not extensive in the majority of these. The assumption—sometimes made clinically—that all patients dying of heart disease have a terminal pneumonia was not confirmed. Hemosiderosis was a frequent finding, being present in all patients with a history of hemoptysis, in 9 of 10 patients with pulmonary infarction, and in 10 of 13 with mitral stenosis.

Pulmonary infarction, a complication in 20% of the cases which was usually not diagnosed clinically, was found to involve the right lung in 9 of 10 cases and the right lower lobe in 7 of the 9. This preferential localization of pulmonary infarcts was confirmed in another series of 25 patients and would appear to be an important diagnostic clue—one which has proved repeatedly valuable during the past two years since the observation was made.

Advanced pulmonary arteriosclerosis was seen with rheumatic mitral valve disease as well as with other types of heart disease where there was a long history of cardiac failure, especially where associated with some emphysema. Pulmonary arteriolar sclerosis was twice as frequent as pulmonary arteriosclerosis and was severer and commoner with mitral stenosis than with any other type of heart disease. Pulmonary capillary fibrosis was likewise closely linked with mitral valve disease, although it was somewhat less common than arteriolar sclerosis. There was no clear evidence that pulmonary arteriolar sclerosis "protected" the alveoli from pulmonary edema, but there was suggestive evidence that alveolar wall fibrosis and pulmonary capillary fibrosis with narrowing of the capillary lumina might act in this capacity. Commonest of all the vascular lesions in the lung was actually dilatation of the lymphatics which occurred with Grade II to IV severity in 80% of the cases.

Our material served to confirm fully the reported rarity of an isolated left pleural effusion in congestive heart failure.

The histologic abnormalities in the liver were divided into three major groups: (1) congestion with or without cellular atrophy (36%), (2) central necrosis with or without inflammation (24%), and (3) an altered hepatic vasculature or "cardiac cirrhosis" (seen in 16%). In correlating the clinical and pathological features of the 38 cases with these definite structural abnormalities, our findings were as follows.

(*a*) There was no evidence that congestion, central necrosis, or an altered hepatic vasculature were confined to any specific type of heart disease as represented in this series. Congestion with or without atrophy was seen in valvular, coronary, and hypertensive heart disease and combinations thereof. The same was true for central necrosis and an altered hepatic vasculature. The liver from one of two patients with tricuspid stenosis showed central congestion with atrophy, and that from the other case showed an altered hepatic vasculature. Previous studies by one of us with Castleman and White [4] indicated the high incidence of an altered vascular pattern or "cardiac cirrhosis" in chronic constrictive pericarditis.

(*b*) There was no relationship of hepatic changes to age of the patient and probably no relation to sex, although males appeared more prone than females to have central necrosis.

(*c*) There was a general but not complete correlation of duration of symptoms and signs of heart failure with type or hepatic change. Thus 22% of the cases with predominant congestion had had such symptoms and signs for three years or more (all but one of these showed associated atrophy); the figure was 33% for those with central necrosis, and it rose to 75% for those with an altered hepatic vasculature.

(*d*) As noted, the heaviest livers usually but not invariably showed minimal or no histologic changes, and the next heaviest were those whose sections showed central necrosis. The smallest liver weights were chiefly found among patients with an altered hepatic vasculature.

(*e*) We were unable to confirm statements in the literature correlating central necrosis with shock.[5,6] The incidence of shock present for 4 hours or more prior to death was almost the same in the three groups with congestion (39%), central necrosis (42%), and an altered hepatic vasculature (50%), and indeed the largest number of patients with shock greater than 24 hours in duration was to be found in the group with only congestion or congestion with atrophy. Central necrosis likewise could not be correlated with the presence of associated pneumonia, although it might be pointed out that one-third of those with central necrosis also had pulmonary infarction. There was no satisfactory correlation of central necrosis with pulmonary alveolar or pulmonary vascular disease or with the use of morphine, mercurial diuretics, or anticoagulants. We did not find central necrosis to be as frequent an accompaniment of cardiac failure as reported by Sherlock[7] and do not believe postmortem material to be as misleading in this regard as she suggests, providing tissue changes are evaluated by experienced pathologists. We would tend to agree with one of the conclusions of Ellenberg and Osserman[6] that central necrosis is "the end result of any prolonged vasospasm, anoxia, or circulatory insufficiency."

(*f*) There was no significant difference in the heart weights in the three groups.

That the hepatic changes we have described have a very real importance in the total picture of congestive heart failure is apparent to us, and indeed the hepatic changes were severe enough in some cases to have been the chief cause of death. While it is not the purpose of the presentation to correlate laboratory tests with pathological findings, it might be pointed out that the prothrombin level taken on one of the patients with Grade III central necrosis with congestion was only 8% the day before death—and he had received no anticoagulants. Such extensive liver-cell damage not only interferes with essential functions in carbohydrate and protein metabolism, including blood clotting, but also provides a large volume of necrotic tissue which may liberate factors injurious in themselves. We suspect that a considerable degree of acute change may be reversible. An example of this appears to have been provided by one patient whose prothrombin level fell to 10% during an episode of severe cardiac failure, with return to normal prothrombin levels with improvement in cardiac failure. At autopsy, a year later, both acute and chronic changes were present.

Venous and capillary engorgement and edema were common in the gastrointestinal tract, notably in the stomach and small intestine. This "water-logging" may play a role in the almost universal postprandial abdominal distress experienced by patients with severe congestive heart failure and may interfere both with motility and with the liberation of digestive secretions and the absorption of food products.[8] Unexpected was the high (at least 8%) incidence of active duodenal ulcers, an incidence over twice that found in routine autopsy statistics at this hospital.

Splenic abnormalities included dilatation of the sinusoids and fibrosis of the red pulp; the last of these when severe appeared closely related to the duration of cardiac failure. The study also reemphasized that congestive heart failure itself is one of the common causes of splenomegaly.

A true nephrosis was seen only three times, and in these three patients it was not possible to incriminate the use of mercurial diuretics. As a group, those patients receiving extensive mercurial therapy showed little evidence of harm, although a mercurial nephrosis was suspected in two other patients. The most frequent renal abnormalities were dilatation of the glomerular spaces and convoluted tubules and edema of the interstitial tissue. Arteriolar disease of the kidneys (and other abdominal organs) was closely related to the presence of hypertension.

As regards the adrenal glands it was found that hyperplasia as determined by microscopic examination of the adrenal cortex occurred in a minority (one-third) of the cases in the series, although total adrenal weights were increased in 68% of the series. Chronic and acute passive congestion of the adrenal medulla were frequent.

The bone marrow was of interest in that erythroid hyperplasia was found in 60% of the cases. That this was the commonest aberration in the bone marrow would tend to support the concept of an increased production of red cells by the marrow under the stimulus of hypoxia in chronic cardiac failure.

In the lower genitourinary tract vascular engorgement and edema of the prostate and testes were the rule, and there was some evidence for reduced spermatogenic activity. These observations would fit with the well-known tendency to obstruction to the bladder neck by prostatic enlargement during cardiac failure and with reduction in sexual potency and fertility complained of by patients with cardiac decompensation.

Summary

A group of 50 cases with congestive heart failure of 48 hours' or more duration are reviewed with a correlation of clinical and pathological features. The latter are given chief emphasis in this description, and the range of gross and microscopic tissue changes are analyzed as to frequency and intensity of pathologic changes encountered. The diffuse nature of organ abnormalities seen is commented upon, and significant extracardiac alterations of function resulting are indicated, notably as found in the lung and liver and to a smaller extent in other tissues. A high incidence of embolic events is particularly observed.

Suite 1811, 122 S. Michigan Ave. (3).

REFERENCES

1. Friedberg, C. K.: Diseases of the Heart, Philadelphia, W. B. Saunders Company, 1949, p. 521.

2. McPeak, E. M., and Levine, S. A.: Preponderance of Right Hydrothorax in Congestive Heart Failure, Ann. Int. Med. 25:916, 1946.

3. Lindsay, S., and Knorp, W. F.: Primary Systemic Amyloidosis, Arch. Path. 39:315, 1945.

4. Paul, O.; Castleman, B., and White, P. D.: Chronic Constrictive Pericarditis: A Study of 53 Cases, Am. J. M. Sc. 216:361, 1948.

5. Clarke, W. T. W.: Centrilobular Hepatic Necrosis Following Cardiac Infarction, Am. J. Path. 26:249, 1950.

6. Ellenberg, M., and Osserman, K. E.: The Role of Shock in the Production of Central Liver Cell Necrosis, Am. J. Med. 11:170, 1951.

7. Sherlock, S.: The Liver in Heart Failure: Relation of Anatomical, Functional, and Circulatory Changes, Brit. Heart J. 13:273, 1951.

8. Altschule, M. D.: Physiology in Diseases of the Heart and Lungs, Ed. 2, Cambridge, Mass., Harvard University Press, 1954, p. 202.

Persistence of Bacterium Tularense in Man in the Absence of Serious Clinical Illness

LT. CMDR. EDWARD A. CARR Jr. (MC), U. S. N. R., and PAUL J. KADULL, M.D., Fort Detrick, Frederick, Md.

Certain pathogenic micro-organisms, e. g., Salmonella typhosa, persist in apparently healthy humans, especially on mucosal surfaces, without producing clinical symptoms. Although Foshay [1] postulated in 1936 that Bacterium tularense may persist in apparently well persons, clinical examples are not common. The following case, in which symptoms were minimal, is presented as an interesting example.

Report of a Case

A 26-year-old white male bacteriologist was first seen as a patient on April 13, 1955. He stated that in August or September, 1952, he had received a single injection of 0.5 or 0.75 ml. of Foshay's tularemia vaccine, which had caused a severe local and systemic reaction. No further injections of the vaccine were given.

For about two years thereafter, he had worked with B. tularense under hazardous conditions. The patient stated that on several different occasions during this interval he had had strongly positive reactions to tularemia skin tests and that his serum had agglutinated B. tularense in a dilution titer of at least 1:160. Unfortunately, detailed confirmation of these data were not available. At no time in his life had the patient experienced known clinical tularemia, to the best of his recollection.

About March 30, 1955, the patient developed a small slightly raised red pruritic lesion over the metacarpophalangeal joint of the right ring finger and mild soreness in the right axilla. The lesion appeared to subside and the axillary tenderness diminished over a period of several days, but then the lesion and tenderness reappeared. Two weeks elapsed before the patient, an intelligent scientific worker familiar with tularemia, seriously considered the possibility that he might have tularemia. The hand lesion and axillary soreness were the only manifestations of his illness; no other signs or symptoms were noted. It was at this point that the patient was first observed.

Physical Examination.—Physical examination revealed a young man in apparent good health. The temperature was 98.0 F orally. There was a slightly elevated red nontender area, about 0.5 cm. in diameter, overlying the metacarpophalangeal joint of the right fourth finger. This area showed slight central dimpling but no ulceration. A soft grape-sized freely moveable nontender right axillary node and a similar but smaller left axillary node were felt. No inguinal nodes were felt. The pharynx, lungs, and abdomen were normal. The serum agglutinated B. tularense to a dilution titer of 1:160, with no cross agglutination of Brucella.

Course and Treatment.—Despite the patient's exposure to B. tularense, and in view of the innocuous appearance of the lesion, a diagnosis of tularemia was questioned, and treatment likely to eradicate common skin pathogens seemed advisable. Procaine penicillin, 600,000 units I. M. daily, and local heat were prescribed.

Two days later sorensss in the left axilla was slightly more pronounced, but otherwise the patient remained asymptomatic. His oral temperature was 98.4 F. The hand lesion and axillary adenopathy showed no change. The white blood cell count was 6750 per cubic millimeter, with neutrophils 52%, lymphocytes 45%, and eosinophils 3%. The sedimentation rate (Wintrobe) was 6 mm. in one hour. The urine was normal except for a trace of albumin. The chest x-ray was normal except for several punctate calcified perihilar lesions. On April 13, two blood cultures were placed on glucose-cystine-blood agar. They remained sterile for 30 days.

The patient picked at his hand lesion, scarifying it slightly, and obtained a small amount of yellowish-white material, which was plated on glucose-cystine-blood agar. In three days, approximately 12 colonies, grossly typical of B. tularense, were observed. The identity of the organism was confirmed by microscopic examination of the stained smear and slide agglutination. The strain to which the patient had been exposed in his work was the Schu-SD strain, known to be highly virulent for several species, including man.

Submitted for publication March 11, 1957.

From the U. S. Army Chemical Corps. Present address of Dr. Carr: Department of Pharmacology, University of Michigan, Ann Arbor, Mich.

Immediately after the cultures had been identified, on April 15, penicillin treatment was stopped. The patient had received a total of 1,200,000 units of penicillin. A course of streptomycin, 0.5 gm. intramuscularly twice daily, and chlortetracycline (Aureomycin), 0.5 gm. orally every six hours, was ordered. Through a misunderstanding, he failed to report for streptomycin the first three days. He received a total of 6 gm. of streptomycin and 18 gm. of chlortetracycline over nine days.

The patient continued to feel well. By April 18 axillary tenderness was gone, and by April 21 the red papular area had faded considerably. The patient was observed several times thereafter and remained well. At the last visit, on July 27, a small pale macule remained at the site of the lesion and the right axillary node remained palpable. Except for the slight scarification produced by the patient, there was never any ulceration.

Serum agglutinin titers for B. tularense were 1:160 on April 21, May 4, May 12, and May 20 and 1:320 on July 27. An intradermal test with a 1:1,000,000 dilution of Foshay's vaccine on May 23 was negative in 48 hours. On May 25, a 1:100,000 dilution was used and gave a positive result in 48 hours.

Comment

A few other instances of bacteriologically proved tularemia with relatively little clinical illness have been reported. Foshay et al.[2] obtained a positive culture from a skin ulcer in a patient with only moderate symptoms. Green and Eigelsbach[3] obtained positive throat cultures on the 3d, 9th, and 17th days after exposure in the case of a technician who had pipetted bacterial suspensions, with consequent pharyngeal ulcers. The patient had a sore throat and spent a short time in bed but was afebrile throughout her illness. She had had a previous attack of tularemia. Howe[4] obtained a positive culture from a patient with ulceroglandular tularemia. The patient was afebrile until streptomycin treatment was started. She then developed a fever attributed to allergy to streptomycin.

All three of the above patients had previously received tularemia vaccination.

Francis reported two attacks of tularemia, 29 months apart, in a laboratory worker. The second attack was manifested by "a papule engrafted on a crack on the finger from which *Bacterium tularense* was isolated by guinea pig inoculation." There was also a lymphadenitis involving the epitrochlear and axillary glands of the same arm but an absence of fever or other notable constitutional symptoms.[5] Francis[6] also described five episodes of tularemia in himself. In the second and fourth of these, 29 months and 13 years, respectively, after the initial bout, positive cultures were obtained from the lesion, a small red papule with a 2 mm. slough, yet the patient had no fever, continued working, and felt only mildly ill. The B. tularense organisms isolated from the lesion killed guinea pigs. Foshay[7] isolated B. tularense, virulent for guinea pigs, from a moderately inflamed bursa of a patient who was, at the time, free of systemic symptoms but who had been seriously ill with tularemia during the preceding months.

Three aspects of the above group of cases appear to merit comment. First, the evidence suggests that in order for B. tularense to be present in human tissues without producing marked clinical evidence of its presence the patient probably had undergone some form of partial immunization—deliberate vaccination, previous clinical attack with or without complete recovery, or, possibly, repeated small exposures, as in the laboratory. The ability to tolerate B. tularense in these cases appears to lie more in increased host resistance than decreased virulence of the bacteria. This is consistent with the results of Coriell's controlled studies,[8] in which vaccinated mice, challenged with B. tularense organisms sufficient to kill unvaccinated mice, remained apparently well yet yielded the organism from small necrotic areas in their tissues when killed. The organisms isolated from the immune mice had not lost their virulence for guinea pigs.

Secondly, as it is impossible to predict when immunity might be overcome by persistent organisms, it seems advisable to treat all tularemic patients, preferably with the bactericidal drug streptomycin. Coriell[8] showed that the immunity of mice could be

overcome if the animals were challenged with a large enough number of organisms.

Lastly, the existing immunity which allows patients to sustain a very mild infection is not always associated with a strong secondary agglutinin response after the mild infection. Green and Eigelsbach[3] noted this. The agglutinin titer failed to rise significantly in Francis' own case after his two very mild episodes with positive cultures. In our patient the highest titer obtained was 1:320 in several months of serological follow-up.

Summary

Bacterium tularense was isolated from a minimal lesion in a man with immunological evidence of prior tularemic infection. The lesion was two weeks old at the time of isolation, and the patient never developed systemic symptoms.

Virulent B. tularense may persist in man and produce only minimal signs of clinical illness if partial immunity has been established in one of several ways. Although such patients cannot be classified as completely asymptomatic carriers, their freedom from any serious symptoms may be striking.

Even in the absence of serious illness, the presence of B. tularense in the tissues warrants chemotherapy.

The very few adequately studied cases suggest that a strong secondary antibody response to reinfection with B. tularense is sometimes absent.

Department of Pharmacology, University of Michigan, Ann Arbor, Mich.

REFERENCES

1. Foshay, L.: in discussion on Blackford, S. D., and Smith, D. C.: Prolonged Virulence of B. Tularense in Human Tissue: Case Report, South. M. J. 29:1062 (Nov.) 1936.

2. Foshay, L.; Hesselbrock, W. H.; Wittenberg, H. J., and Rodenberg, A. H.: Vaccine Prophylaxis Against Tularemia in Man, Am. J. Pub. Health 32:1131 (Oct.) 1942.

3. Green, T. W., and Eigelsbach, H. T.: Immunity in Tularemia: Report of 2 Cases of Proved Reinfection, Arch. Int. Med. 85:777 (May) 1950.

4. Howe, C.; Coriell, L. L.; Bookwalter, H. L., and Ellingson, H. V.: Streptomycin Treatment in Tularemia, J. A. M. A. 132:195 (Sept. 28) 1946.

5. Francis, E.: Tularemia. Second Ludvig Hektoen Lecture of the Billings Foundation, Proc. Inst. Med. Chicago 6:27, 1926.

6. Francis, E.: Immunity in Tularemia, Tr. A. Am. Physicians 51:394, 1936.

7. Foshay, L., and Mayer, O. B.: Viability of Bacterium Tularense in Human Tissues, J. A. M. A. 106:2141 (June 20) 1936.

8. Coriell, L. L.; Downs, C. M., and Clapp, M. P.: Studies on Tularemia: III. Immunization of Mice, J. Immunol. 56:245 (July) 1947.

Paroxysmal Nocturnal Hemoglobinuria with Cirrhosis and Hemosiderosis

Report of a Case with Autopsy Findings

ARTHUR E. DAVIS Jr., M.D., Minneapolis

Paroxysmal nocturnal hemoglobinuria (PNH) was first described by Paul Strübbing, in 1882.[1] His paper was a classic in clinical investigation and description, but his contribution remains little known. In 1911, Marchiafava [2] described a case of acquired hemolytic anemia, "Widal-Abrami type," which was characterized by large amounts of hemosiderin in the urine. Nocturnal hemoglobinuria was not noted. It was not until 1928 that Marchiafava published his article on a clinical syndrome characterized by chronic hemolytic anemia associated with paroxysmal nocturnal hemoglobinuria.[3] In 1931, Micheli also described the syndrome.[4] Although independently described by Marchiafava and Micheli, the disease is now known as the Marchiafava-Micheli syndrome.

In 1937, Ham demonstrated that the prime abnormality rests in the red cells, not in the patient's serum.[5] The red cells are hemolyzed as a consequence of slight lowering of the pH within the physiological range. Carbon dioxide retention during the slow and shallow respirations of sleep was implicated. Ham also demonstrated the presence of a thermolabile factor, which is present in all human serums and is essential to the hemolysis but has no effect on normal erythrocytes.[6]

In 1952, Clapp demonstrated that at least two serum factors were responsible in PNH.[7] One of the factors is thermolabile and magnesium-dependent, and the other is an adsorbable globulin. In 1953, Crosby published a most important and complete work on the blood factors and mechanisms.[8] He states that there are two hemolysins opposed by two inhibitors and that the system is in a delicate balance that can be shifted by very slight changes in blood pH and other factors.

Clinically, the onset of PNH may be gradual or abrupt. The disease usually occurs in the third or fourth decade, although cases in older and younger age groups have been reported. If the onset is gradual, symptoms of anemia are experienced before gross hemoglobinuria is evident. No hereditary tendency or sex predilection has been reported.

There is little material in the literature concerning the autopsy data. Approximately 10 cases have been reported, and many of these lack detailed descriptions. The commonest pathological findings may be summarized as follows: (1) enlarged kidneys showing marked hemosiderosis of the proximal convoluted tubules, moderate hemosiderosis of the distal convoluted tubules, and usually no pigment deposit in the collecting tubules (several cases also had hydropic degeneration of the proximal convoluted tubules); (2) hepatomegaly with central zonal necrosis; (3) moderate splenomegaly; (4) venous thromboses of both the portal and the systemic circulation.

The following case report emphasizes the usual histopathological findings in PNH and is unusual in that there is an associated cirrhosis with hemosiderosis. It is realized that the term "hemochromatosis" is broad

Submitted for publication Feb. 15, 1957.

Present address: The St. Cloud Hospital, St. Cloud, Minn.

and nonspecific and that in many occasions the differentiation between true hemochromatosis and hemosiderosis is not possible. However, this case fulfills the histopathological requirements for a diagnosis of exogenous hemochromatosis. Secondary or exogenous hemochromatosis has been defined by Aufderheide, et al.[9] as a consequence of anemia, blood transfusions, or both and characterized by increased hepatic and total body iron and unequivocal portal cirrhosis.

The association of PNH and secondary hemochromatosis was first described by Heffernan and Jasevon, in 1955.[10] The findings at autopsy differed from the usual case of PNH in that there was no splenomegaly, hepatic central zonal necrosis, or venous thrombosis. The authors point out that in classical PNH renal hemosiderosis is associated with depletion or normal iron stores, whereas in both exogenous and endogenous hemochromatosis the body tissues are saturated with iron but the kidney contains little or none. The daily loss of iron in PNH is up to 5 mg., but despite this the patients are usually not markedly iron-deficient. Perhaps there is a compensatory increased absorption of iron from the gastrointestinal tract as postulated in hemochromatosis and other anemias. A simple analogy is the salvage of iron in internal bleeding and hemolysis as contrasted to the loss of iron in external hemorrhage. In the case to be presented here the large amount of iron given by blood transfusion plus the intravascular hemolysis can explain the hemosiderosis.

Clinical Summary

The case is that of a 61-year-old white male gear cutter who was first admitted to the Veterans' Administration Hospital, Minneapolis, in June, 1951, because of progressive weakness of six month's duration.

Physical examination revealed a well-developed well-nourished, white man who appeared pale. The liver and spleen were palpable, as well as a few shotty nodes in the left axilla.

Admission Laboratory Examination

Hemoglobin, 4.8 gm. per 100 cc.; RBC, 1,800,000 per cubic millimeter; WBC, 2000 per cubic millimeter, with 56% polymorphonuclear leukocytes, 39% lymphocytes, 4% monocytes, and 1% eosinophils. Platelet count, 80,000 per cubic millimeter; sedimentation rate, 100 mm. in one hour; hematocrit, 18%. Mean corpuscular diameter, 8μ; mean corpuscular volume, 100 cu.μ; mean corpuscular hemoglobin, 26.6 $\mu\gamma$, mean corpuscular concentration, 26.6%. Reticulocyte count, 15.3%; bleeding time, three minutes; clotting time, eight and one-half minutes.

Results of serologic test for syphilis were negative; fragility test, within normal limits; Coomb's test, negative; clotting tests, normal; cold agglutinins, negative. Total proteins were 6.1 gm. per 100 cc.; urinalysis, negative, except for 1+ albumin. Liver function studies were within normal limits. Stool was negative for occult blood. Four-day fecal urobilinogen assay revealed 680 mg. per day. Gastric analysis revealed 40 degrees of free acid. Findings on ECG were normal. Lymph node biopsy revealed benign hyperplasia. Bone marrow biopsy exhibited extreme normoblastic hyperplasia.

Hospital Course

In view of the marked leukopenia and evidence of hemolysis, the probable diagnosis of lymphoblastoma was made, and the patient was given seven blood transfusions. It was noted a few days after admission that the morning urine specimen was dark red in color and positive for hemoglobin and hemosiderin. The patient stated that this was first noted in March, 1951, and that it cleared up within two days. Since that time he had noted some dark urine almost every day, most frequently in the morning after arising. He never noted it to be red during the day. There were no complaints of fever, chills, dyspnea, or abdominal pain. A trial was made of reversing his sleeping habits, which also reversed his hemoglobinuria; that is, his nighttime waking urines were negative for hemoglobin, but those after a daytime sleep showed hemoglobin. A modified Ham test for acid hemolysis was positive. The diagnosis of PNH was made, and he was followed in the outpatient department. His illness gradually became more severe. Transfusions were required for maintenance. The hepatosplenomegaly persisted.

In September, 1951, he was treated for intestinal obstruction with good results. It was noted the patient's skin had a bronze cast. In November, 1951, he became jaundiced, and the liver function tests were abnormal. In April, 1952, he developed antibodies to Kell and C antigens, and his indirect Coomb's test had become positive. He also developed pancytopenia, with a white blood cell count around 1500 per cubic millimeter and platelets around 50,000 per cubic millimeter. He was

given a course of corticotropin (ACTH), resulting in an elevation of the white blood cell count. He was given a course of cortisone, but this failed to influence the blood picture. In September, 1952, a splenectomy was performed. The spleen weighed 500 gm. and contained excessive iron. A liver biopsy at surgery revealed hemosiderosis but no cirrhosis. After splenectomy the patient's leukopenia improved, but his anemia and thrombocytopenia persisted. In December, 1952, the patient developed fluid retention, which was treated with only moderate success.

The last admission, on May 16, 1955, was for further transfusion therapy. The patient was jaundiced, but in no acute distress. The abdomen was distended. The liver was palpable 4 to 5 cm. below the costal margin and nontender. Chest x-ray and bilateral retrograde pyelography were normal. Hemoglobin was 6.8 gm. per 100 cc.; platelets, 8700 per cubic millimeter; serum proteins, normal; BUN, 17.2 mg. per 100 cc., and there was albuminuria. Urea clearance was 93% and 96% of normal. P. S. P. showed 4% excretion in 15 minutes, with a total of 9% in 30 minutes. Suddenly, on May 24, 1955, the patient had a large coffee-ground emesis followed by tarry stools. It was assumed the patient was bleeding from esophageal varices secondary to cirrhosis of the liver, and 4 units of whole blood was given. Despite therapy, his condition gradually deteriorated, and he died in hepatic coma on July 1, 1955. He received a total of 166 units of whole blood, or 40.2 gm. of iron, during the four years of treatment.

Final Clinical Diagnoses

The final clinical diagnoses were as follows: (1) paroxysmal nocturnal hemoglobinuria with (*a*) hemolysis, (*b*) hepatomegaly, and (*c*) ascites; (2) intestinal hemorrhage due to undetermined cause; (3) incisional hernia.

Autopsy Findings

Gross Findings: There was marked jaundice and there were scattered petechial hemorrhages over the hands and arms. The peritoneal surfaces were hemorrhagic and covered with fibrin strands. There were old fibrous adhesions in the right upper and lower quadrants. Twelve liters of dark-brown watery fluid was found in the peritoneal cavity. There was approximately 50 cc. of amber fluid in each pleural cavity.

The heart weighed 300 gm. and was not remarkable. The right lung weighed 445 gm.; the left, 420 gm. There was minimal pulmonary edema.

The liver weighed 2100 gm. The surface was covered with scattered yellowish plaques that measured 3 mm. in thickness and up to 8 mm. in diameter. The parenchyma was deep reddish-brown. The portal connective tissue was prominent, producing a diffuse nodularity. The central veins were accentuated. Dilated veins throughout the parenchyma were thrombosed and surrounded by necrotic parenchyma. The gallbladder was scarred. The hepatic artery and the portal vein were normal.

The spleen was absent. The pancreas weighed 70 gm. and was deep yellowish-brown. The right adrenal weighed 10.5 gm.; the left, 11 gm. Both exhibited deep brownish flecking throughout both the medulla and cortex.

The right kidney weighed 180 gm., the left, 198 gm. They were deep brown, and the capsules were not adherent. The external surfaces were smooth, except for two clear fluid cysts in the superior pole of the left kidney. The cortex was of normal thickness and exhibited a deep brownish pigmentation. The medullary portions were grossly devoid of pigment, producing a sharp contrast at the corticomedullary junction. The rest of the urinary tract was not remarkable. No esophageal varices were found. There was a shallow ulcer on the posterior superior wall of the duodenum that appeared fresh.

Microscopic Findings (Hematoxylin-and-Eosin and Iron Stains): Microscopically, the heart exhibited moderate fibrosis and hemosiderosis. There was minimal coronary artery atherosclerosis. There was moderate congestion of the lungs, with numerous pigment-filled macrophages in the alveolar spaces.

The liver exhibited a moderate to marked collagenous and hyaline capsular thickening. The normal parenchymal architecture was almost entirely obliterated by three separate processes. There was primarily a portal cirrhosis characterized by a moderate to marked relative increase in the portal connective tissue, with division of the parenchyma into irregular small nodules. There was an associated proliferation of bile ducts and a moderate increase in the round-cell infiltrate. The second prominent change was characterized by an extreme deposit of hemosiderin within the hepatic cells, within the reticuloendothelial system, within the bile-duct epithelium, and within the portal connective tissue. The third process consisted of central zonal necrosis with asso-

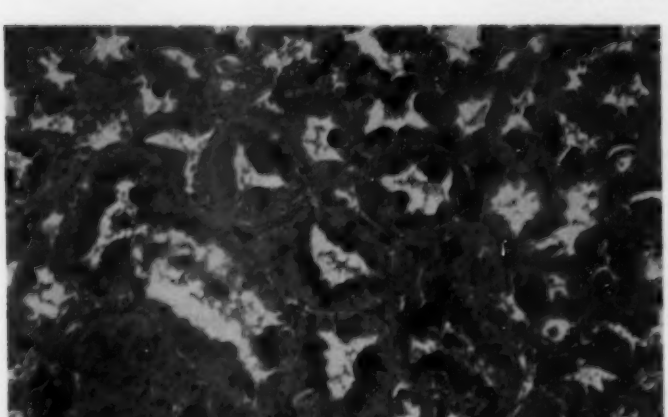


Fig. 1.—Marked hemosiderosis of the proximal convoluted tubules of the kidney. Hematoxylin and eosin stain; reduced ⅓ from mag. × 100.

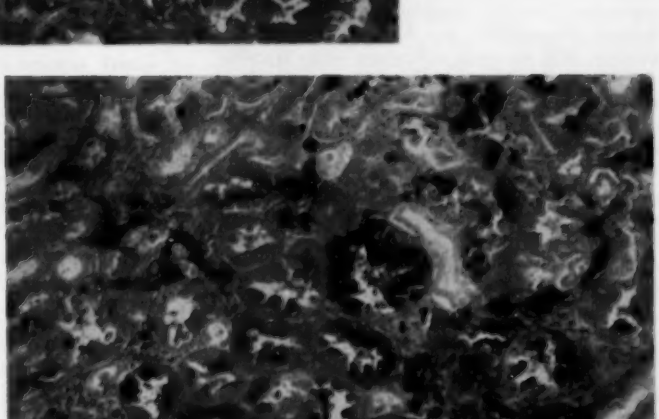


Fig. 2.—Marked hemosiderosis of the proximal convoluted tubules of the kidney. Iron stain; reduced ⅓ from mag. × 100.

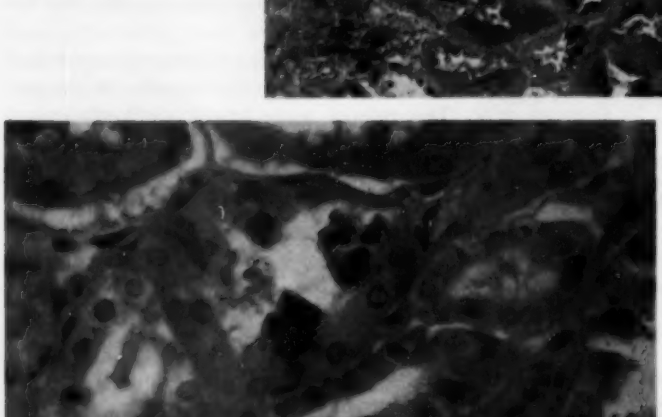


Fig. 3.—The proximal convoluted tubules exhibit a heavy deposition of iron, while the distal convoluted tubules are almost devoid of iron. Hematoxylin and eosin; reduced ⅓ from mag. × 400.

Fig. 4.—Liver. There is an organized venous thrombosis in the right lower corner. The circumscribed dark areas represent fresh parenchymal necrosis. A very heavy deposition of hemosiderin has produced the diffuse, heavy, granular mottling. The light bands of tissue coursing throught the section represent the increased portal connective tissue. Hematoxylin and eosin; reduced ⅓ from mag. × 6.

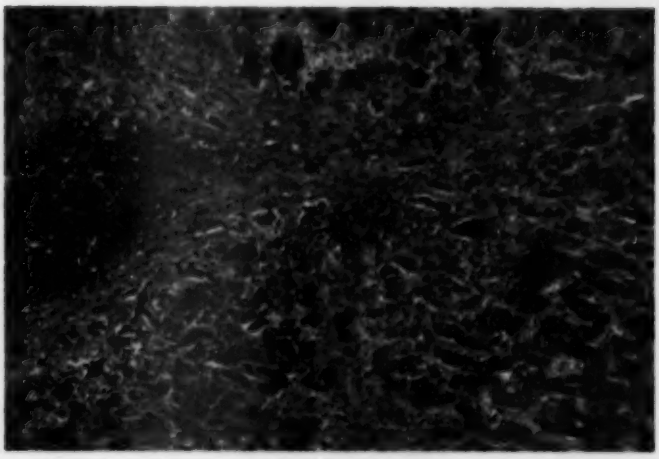


Fig. 5.—Liver. There is an area of necrosis on the right. Note the hemosiderosis and cirrhosis. Hematoxylin and eosin; reduced ⅓ from mag. × 100.

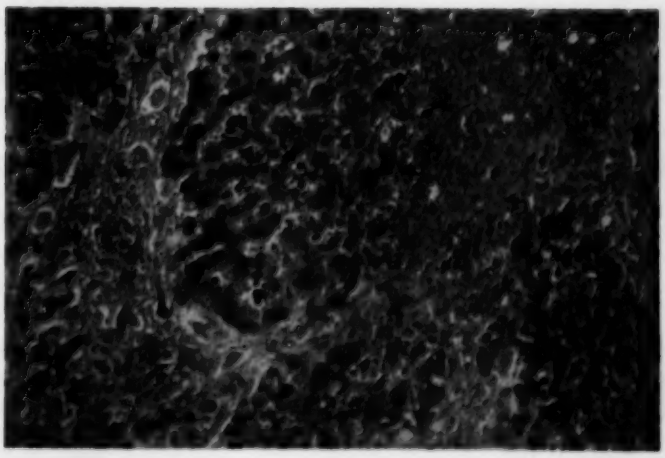


Fig. 6.—Liver. The area of hemorrhage in the left lower corner indicates recent parenchymal necrosis. The iron stain clearly demonstrates the extreme hemosiderosis. Iron stain; reduced ⅓ from mag. × 100.

Fig. 7.—Hemosiderosis of the adrenal gland. Iron stain; reduced ⅓ from mag. × 100.

Fig. 8.—Cellular detail of the hemosiderosis in the pancreas. Iron stain; reduced ⅓ from mag. × 400.

ciated venous thrombosis. The process was extensive, and although all stages of necrosis and repair were present, most of the changes were acute in nature. The process varied from a small amount of necrosis immediately surrounding the central vein to areas that had spread out to involve the entire lobule, leaving only large areas of necrosis and hemorrhage enclosed by fibrous portal connective tissue. In all sections studied there were numerous venous thromboses exhibiting all stages of organization. Some were old, completely occluding the lumen, and hyaline. Others were more recent and exhibited only characteristic zoning changes.

The pancreas exhibited a heavy deposit of iron within the islet and acinar epithelium, the trabecular connective tissue, and to a much smaller extent the ducts. There was also a moderate amount of iron in the surrounding fat and connective tissue which appeared to be within the macrophages. There was a very heavy deposition of iron pigment within the adrenals. Deposition was primarily within the reticulum and connective-tissue stroma, but also with extensive involvement of the epithelium of both the cortex and the medulla. As in the pancreas, there was also an overflow into the surrounding connective tissue and fat.

The kidneys exhibited an extreme deposition of iron, primarily within the tubules. The process was severest within the proximal convoluted tubules, to a smaller extent within the loop of Henle and distal convoluted tubules, and minimally within the collecting tubules. The tubular epithelium, although in many instances almost completely replaced by iron, exhibited no evidence of degeneration or damage. Under low power, this extreme involvement of the tubular structures within the cortex and the paucity of iron within the medulla was most striking. Associated with this were moderate arteriosclerotic changes.

There was moderate to marked atrophy of the testicles, with an associated heavy deposition of iron within the testicular epithelium as well as in the surrounding connective tissue. The duodenum exhibited an active ulcerative process with minimal reaction. There was a moderate amount of iron within the epithelium of the mucosa.

The lymph nodes exhibited hemosiderosis. The bone marrow was of approximately normal cellularity, with a mild relative normoblastic hyperplasia. There was a marked increase in the particulate iron.

The pituitary exhibited a moderate deposition of iron within both the reticulum and the parenchymal cells. The brain exhibited perivascular pseudocalcinosis.

The final anatomic diagnoses in this case were listed as follows: (1) paroxysmal nocturnal hemoglobinuria with, (*a*) Laennec's portal cirrhosis of the liver, (*b*) central zonal necrosis of the liver, secondary to multiple venous thromboses, (*c*) extreme hemosiderosis of both the reticuloendothelial and the epithelial components of all organs examined; (2) myocardial fibrosis (mild); (3) pulmonary congestion (moderate); (4) duodenal ulcer (acute).

Sections of the spleen and liver that were removed previously were reviewed. The liver exhibited a moderate hemosiderosis, minimal fatty metamorphosis, but no evidence of cirrhosis, necrosis, or thrombosis. The spleen exhibits only minimal hemosiderosis.

Comment

This case illustrates all of the characteristic findings of PNH plus exogenous hemochromatosis with cirrhosis. Perhaps the reticuloendothelial system became saturated by both endogenous and exogenous iron, with a subsequent overflow to involve the epithelium of the body. This overflow includes the epithelium of the hepatic cords, biliary radicles, pancreas, adrenals, testicles, and gastrointestinal tract. The accumulation of iron is adequately explained by four years of intravascular hemolysis plus 40.2 gm. of transfused iron. Although typical changes of portal cirrhosis were present, at no time was there any evidence of diabetes mellitus. Consequently, a relationship to endogenous hemochromatosis cannot be established.

The renal lesions are fascinating in that the altered physiology correlates well with the anatomic changes. Thus the relatively normal appearance of the vessels, glomeruli, distal convoluted tubules, and collecting tubules is reflected by the normal urea clearance test, ability to concentrate maximally, and normal BUN. Saturation of the proximal convoluted tubules by iron is reflected by the marked reduction of the P. S. P. excretion test.

Finally, this case reiterates the relentless course and outcome of this disease and the absence of any significant benefit from the various types of therapy available.

Summary

A case of paroxysmal nocturnal hemoglobinuria (Marchiafava-Micheli syndrome) with hemosiderosis is presented. The literature is briefly reviewed, and the characteristic gross and microscopic autopsy findings are noted. These include (1) enlarged kidneys, showing marked hemosiderosis of the proximal convoluted tubules, moderate siderosis of the distal convoluted tubules, and usually no pigment deposit in collecting tubules; (2) hepatomegaly, with central zonal necrosis; (3) moderate splenomegaly;

(4) venous thromboses of both the portal and the systemic circulation.

The correlation between the histopathology of the renal lesions and the altered renal physiology is discussed.

St. Cloud Hospital, St. Cloud, Minn.

REFERENCES

1. Strübing, P.: Paroxysmale Haemoglobinuria, Deutsche med. Wchnschr. 8:1 and 17, 1882.

2. Marchiafava, E., and Nazari, A.: Nuovo contributo allo studio degli itteri cronici emolitici, Policlinico (sez. med.) 18:241, 1911.

3. Marchiafava, E.: Anemia emolitica con emosiderinuria perpetua, Policlinico (sez. med.) 35: 105, 1928.

4. Micheli, F.: Anemia (splenomegalia) emolitica con emoglobinuria-emosiderinuria tipo Marchiafava, Haematologica I. Arch. 12:101, 1931.

5. Ham, T. H.: Chronic Hemolytic Anemia with Paroxysmal Nocturnal Hemoglobinuria: Study of the Mechanism of Hemolysis in Relation to Acid-Base Equilibrium, New England J. Med. 217:915, 1937.

6. Ham, T. H.: Studies on Destruction of Red Cells: Chronic Hemolytic Anemia with Paroxysmal Nocturnal Hemoglobinuria; Investigation of Mechanism of Hemolysis with Observations on 5 Cases, Arch. Int. Med. 64:1271, 1939.

7. Clapp, M. P.; Williams, M. J., and Mendel, J. L.: Observations on the Hemolytic Mechanism of Paroxysmal Nocturnal Hemoglobinuria: Demonstration of Activity of at Least 2 Serum Factors in PNH Hemolysis, Blood 7:1117, 1952.

8. Crosby, W. H.: Paroxysmal Nocturnal Hemoglobinuria: Relation of Clinical Manifestations to Underlying Pathogenic Mechanisms, Blood 8:769, 1953.

9. Aufderheide, A. C.; Horns, H. L., and Goldish, R. J.: Secondary Hemochromatosis: Transfusion (Exogenous) Hemochromatosis, Blood 8:824, 1953.

10. Heffernan, C. K., and Jaswon, N.: A Case of Paroxysmal Nocturnal Hæmoglobinuria Associated with Secondary Hemochromatosis, a Lower Nephron Nephrosis, and a Megaloblastic Anemia, J. Clin. Path. 8:211, 1955.

Electron Microscopic Observations of Blood Platelets and Fibrin Formation

GLEN B. HAYDON, M.D., San Francisco

The structure of the blood platelet is difficult to study for many reasons, the most important of which is that any manipulation causes changes. In relatively fresh preparations, however, platelets appear as rounded disks, some more oval and others more spherical.[1-3] Platelet processes seem to be formed only after some damage has been done to the platelets or after the environment has been changed. How the processes form is not clear; they develop before fibrin appears and remain separate from the strands of fibrin.[4] There is nothing to suggest that they result from ameboid movement.[5] Until electron microscopic observations of thin sections were made, the internal structure of the platelet was more difficult to study.[6,7] Intact platelets collected early are very small and almost completely electron-opaque. However, some vital-stained preparations examined under the ordinary microscope show two types of mitochondria.[1] Some whole-platelet preparations, when examined in the electron microscope, show large opaque granules in the central portion, the chromomere, and a less opaque peripheral portion, the hyaloplasm.[8] In thin sections the finer structures appear to include paired lamellar structures with some vacuoles, suggestive of Golgi apparatus, and some larger granules, suggestive of mitochondrial origin.[7]

In the present study an attempt was made to collect platelets for electron microscopic examination as rapidly as possible from freshly drawn whole blood in the hope of being able to find and follow progressive changes in their form and their association with the appearance of fibrin in whole blood. The methods differ from those of others who have developed methods for routine studies of platelets in that no anticoagulants were used.[9]

Submitted for publication May 6, 1957.

Instructor, Department of Pathology, Stanford University School of Medicine.

This work was performed during the tenure of a Marie Luise Graham Fellowship.

Methods

Individual preparations were made in a standard manner: a wire loop, previously coated with a Formvar film, was dipped for two to five seconds into a fresh aliquot of venous blood in a silicone-coated test tube, washed rapidly in water, fixed in 2% buffered osmic acid, washed again in water, and dried. The entire procedure was completed in about 30 seconds. The dried Formvar film was then transferred to mounts for electron microscopic examination in the Philips EM 75. Such preparations could be examined in the electron microscope within an hour from the time the blood was drawn.

This basic schedule of preparation was adopted after controlled variations of the steps, especially of the times involved. By varying the time the loop is in the blood and the amount of agitation, marked and inconstant differences in the appearance of the platelets were found.

A series of preparations was made from the blood of each of 25 patients in the clinical laboratory of Stanford University Hospitals. The blood was divided into 1 ml. aliquots in silicone-coated test tubes; one platelet preparation was made from each aliquot. Preparations were made at regular intervals until a clot formed.

Bovine fibrinogen (the supernatant of a 500 mg. per 100 ml. mixture) and thrombin, in concentrations from 0.04 to 1.0 units per milliliter, were mixed in equal portions and treated in a manner similar to samples of fresh whole blood, i. e., similar serial preparations were made.[10]

Results

In all preparations platelets were found. There was considerable variation in the

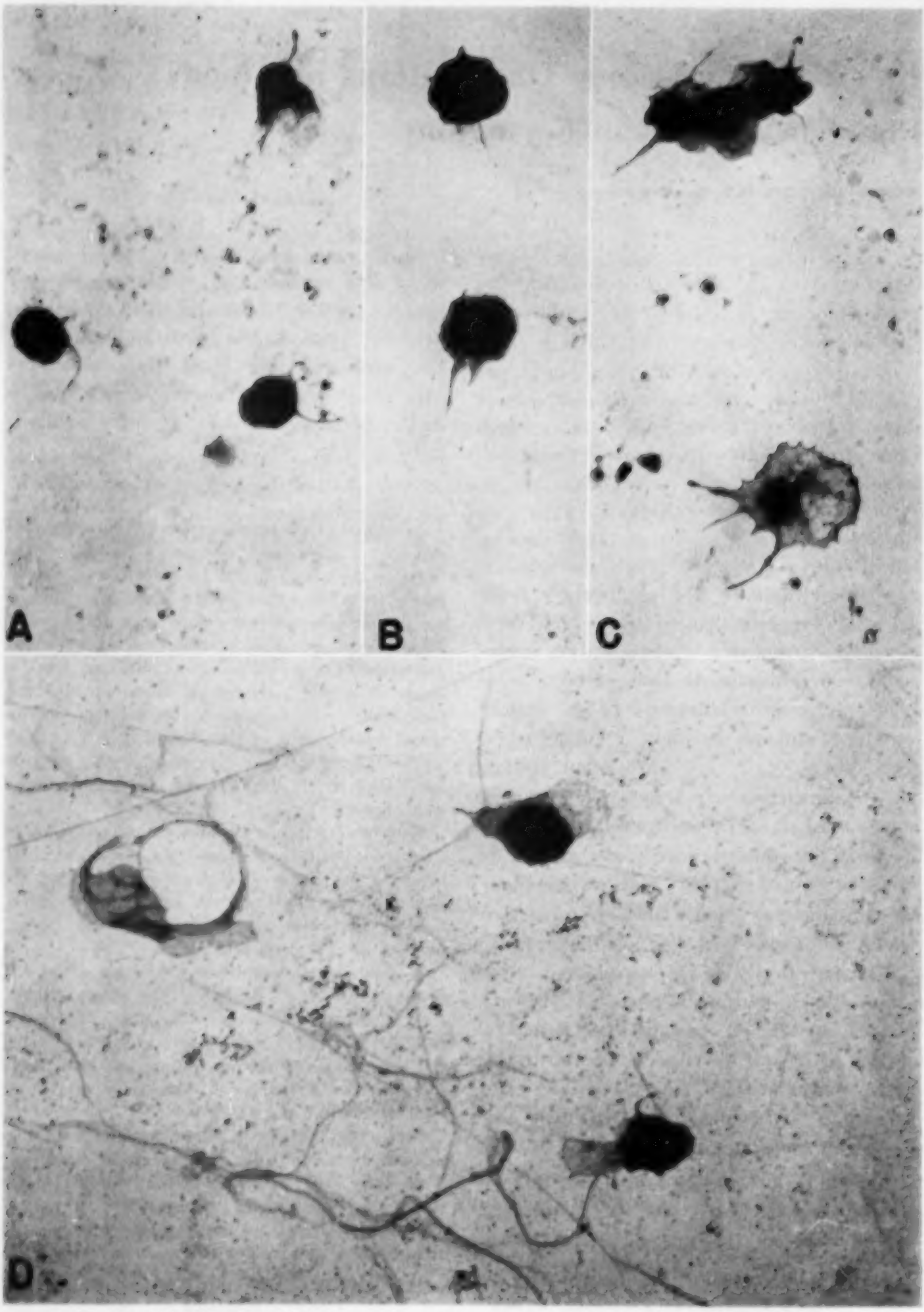


Fig. 1.—Platelets in serial preparations of a sample of blood. *A*, at 3 minutes; *B*, at 6 minutes; *C*, at 9 minutes, and *D*, at 12 minutes. At 12 minutes fibrin is found on the Formvar film distinctly separate from the platelets; reduced 1/12 from mag. × 3500.

appearance of the platelets when the films were left in the blood for longer than five seconds, with or without agitation. Leaving the Formvar film in the blood for longer periods did not increase the total number of platelets collected. The appearance of the platelets was most nearly constant, both on the same preparations and similar preparations, when the film was left in the blood for the shortest period.

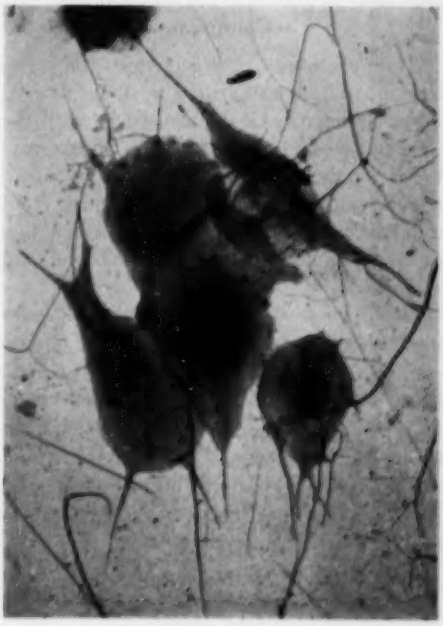
The platelets in the serial preparations showed a progressive change from relatively small compact forms with few processes to larger more spread-out forms with more processes, and the number of platelet clumps increased (Fig. 1). The central portion of the platelet remained intact and was completely electron-opaque in almost all preparations, though a few of the latter preparations in a series included spread-out forms showing some coarse opaque granules. In some preparations—especially those without prompt fixation—the coarse granules of the central portion were found more frequently. Some of the clumped platelets appeared to show a coalescense of the peripheral spread-out portion and more granularity in the central opaque portion. The platelet margin was in some places intact with a number of processes, while in other places the margins were indistinct, suggesting that the content of the platelet was spilling out onto the Formvar film. The substance of the spread-out portion often contained clear vacuoles. The processes were never more than twice as long as the body of the platelet and usually only about the same length. Some of the processes ended in relatively sharp points, but most seemed to have a clubbed end. The substance of the process was almost homogenous.

The preparations from one patient showed very few and markedly atypical platelets. The patient had a pancytopenia of unknown etiology. Besides being scanty, the platelets had short processes which appeared to be broken—no difference could be seen in the central portion.

The later preparations in a series often included strands of fibrin; the time of appearance corresponded to the clotting time of the blood. The fibrin strands at first appeared to be stuck to the Formvar film and were free from the individual platelets on the film (Figs. 1*D* and 2). Small fibrils appeared to coalesce to form larger fibers—the fibers were frayed, and the ends often looked like tassels (Fig. 3). The appearance was identical with that of the bovine fibrin formed in a mixture of fibrinogen and thrombin (Fig. 4).

The larger fibers were electron-opaque and appeared to be stuck to the dense portion of smaller platelets as well as to the film. In many areas the platelet processes and the fibrin strands could be easily distinguished; in no areas were extensions of platelet processes into fibrin strands identified.

Fig. 2.—Platelets and fibrin in a preparation made nine minutes after the blood was drawn. The separation of the fibrin fibers and platelet processes is quite clear; reduced approximately ¼ from mag. × 6500.



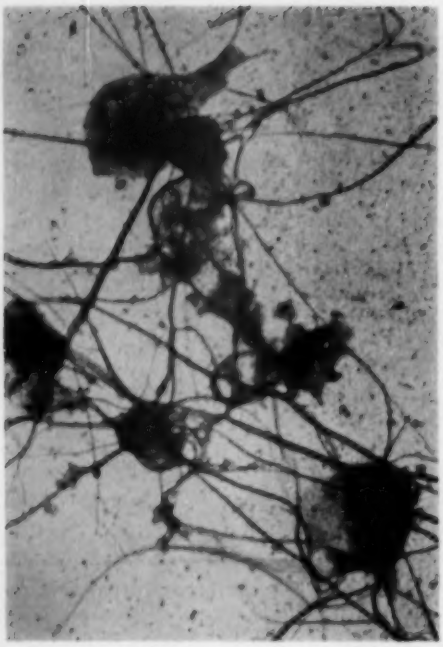


Fig. 3.—Platelets and fibrin in a preparation made at nine minutes after the blood was drawn. The fibrin fibers appear to be stuck to the central portion of the platelets. The fraying of the fibers is well demonstrated, reduced approximately ¼ from mag. × 6500.

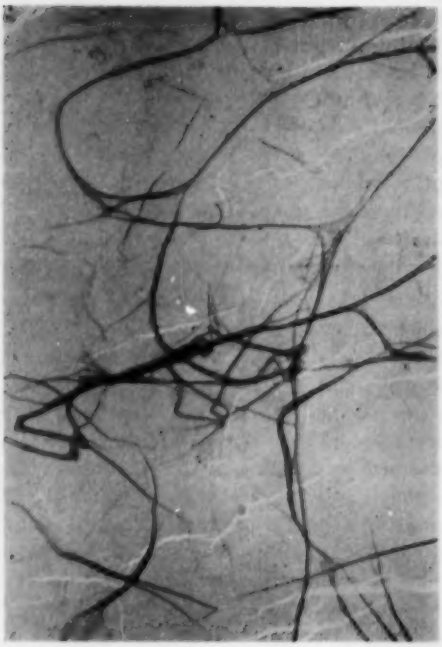


Fig. 4.—Preparation of bovine fibrin, 0.1 units per milliliter of thrombin and fibrinogen mixed in equal portions, made 12 minutes after mixing. The distribution of fibrin is strikingly similar to that found in the preparations made from whole blood; reduced approximately ¼ from mag. × 6500.

Comment

The progressive enlargement of the platelets and the alteration of the surface membrane in some is clearly consistent with the idea that some of the platelets disintegrate before clot formation begins. The fibrin fibrils seem to form at some distance from the platelets—at least on the Formvar film—as well as almost on top of the platelets; in some areas it was possible to discern fibrils forming atop and around platelets with intact membranes; no areas were found to suggest that the platelet processes formed fibrin bundles. This leads to the conclusion that, though some platelets contribute to the precursory chemistry of clot formation, the reaction of thrombin and fibrinogen does not take place at the platelet but rather diffusely in the plasma.

The nature of the platelet processes is not clear. They are not numerous in very fresh preparations, but their number increases as the platelets age. Frequently they have rounded or clubbed ends which appear to be stuck to the Formvar film. These ends may be a stickier portion of the platelet surface; the configuration of some of the processes suggests that the end sticks to the film first and this causes the process to stretch out.

The platelet processes are often clearly distinct from the overlyin fibrin fibers, and their density is different. Nowhere is there a clear suggestion that the fibrin develops from the platelet membrane. The present work differs from that of others, suggesting that the fibrin originates from the platelet membrane in that no additives were used and the preparations were completed within minutes of the time the blood was drawn. The present work strongly suggests that fibrin forms apart from the platelets and

that the fibrin fibers and platelets stick together because of a mutual stickiness. As a fiber passes over the surface of an intact platelet it sticks, the phenomenon being independent of the fibrin formation. One can only speculate about the nature of the stickiness, but perhaps thin sections perpendicular to adjoining surfaces will reveal something of the nature of the mechanisms involved.

In conclusion, the blood platelets contribute both to the chemical components necessary for fibrin formation and to the clot strength by a sticky property. These phenomena appear to be separate properties of platelets. Some platelets disintegrate rapidly, while others remain small and more nearly intact.

Summary

A rapid method for the examination of blood platelets by electron microscopy is described, and selected electron micrographs from a series of blood samples are presented and discussed.

The observed progressive enlargement of the platelets with increased clumping and disintegration of some platelets is consistent with the concept of platelet destruction before clot formation.

Some of the fibrin found in preparations made later in a series was not directly connected to platelets, suggesting that the incorporation of platelets into a network of fibrin occurs after the formation of the fibrin, perhaps by the sticking of the platelets and fibrin strands.

The electron micrographic appearance of human fibrin is similar to that of fibrin formed in a mixture of bovine fibrinogen and thrombin.

The platelets in preparations from the blood of a patient with pancytopenia were morphologically different from the platelets in all of the other preparations.

Department of Pathology, Stanford University School of Medicine, 2398 Sacramento St. (15).

REFERENCES

1. Ralph, P. H.: The Human Platelet, Anat. Rec. 106:234, 1950.

2. Zucker, M. B., and Borrelli, J.: Reversible Alterations in Platelet Morphology Produced by Anticoagulants and by Cold, Blood 9:602, 1954.

3. Bessis, M.: Studies in Electron Microscopy of Blood Cells, Blood 5:1083, 1950.

4. Mann, F. D.; Hurn, M., and Mathieson, D. R.: Platelets as Foci in Coagulation of Blood, Am. J. Physiol. 158:84, 1949.

5. Ferguson, J. H.: Observations on the Alterations of Blood Platelets as a Factor in Coagulation of the Blood, Am. J. Physiol. 108:670, 1934.

6. Bessis, M.: Cytology of the Blood and Blood-Forming Organs, translated by Eric Ponder, New York, Grune & Stratton, Inc., 1956.

7. Rinehart, J. F.: Electron Microscopic Studies of Sectioned White Blood Cells and Platelets, with Observations on the Derivation of Specific Granules from Mitochondria, Am. J. Clin. Path. 25: 605, 1955.

8. Bloom, G.; Gustavson, K. H., and Swensson, A.: On the Reaction of the Thrombocytes to Submicroscopic Particles in Vitro, Acta haemat. 13:57, 1955.

9. Rebuck, J. W.: A Simple Direct Method for the Electron Microscopy of Peripheral Blood Cells, Am. J. Clin. Path. 19:217, 1949.

10. Porter, K. R., and Hawn, C. V.: Sequences in the Formation of Clots from Purified Bovine Fibrinogen and Thrombin: A Study with the Electron Microscope, J. Exper. Med. 90:225, 1949.

The Spleen in Ethionine-Induced Cirrhosis

Its Role in γ-Globulin Elevation

GEOFFREY KENT, M.D.; HANS POPPER, M.D.; ALVIN DUBIN, M.S., and CLARA BRUCE, B.S., Chicago

Splenomegaly and serum γ-globulin elevation are common findings in hepatic disease, especially cirrhosis. The nature of the splenomegaly and the role of the spleen in the serum γ-globulin elevation are still open questions. According to some observers,[1-4] the splenomegaly is chiefly hydromechanical in origin, while others[5-10] feel that it is the result of reticuloendothelial stimulation by toxic processes, perhaps from liver-cell injury. The coarse nodular cirrhosis produced by ethionine with concomitant rise of serum γ-globulin[11] presents a resemblance to human lesions. This experimental hepatic injury provided a tool for the study of the factors responsible for the splenomegaly, portal hypertension, and serum γ-globulin elevation.

Methods

Female Sprague-Dawley rats were kept on a synthetic diet (containing vitamin-free casein, 160 gm.; corn oil, 50 gm.; sucrose, 750 gm.; salt mixture U. S. P., for depletion diet, 40 gm. per kilogram, and adequate vitamin supplements, except for riboflavin, 4 mg., and choline hydrochloride, 30 mg.) to which 0.5% ethionine was added. Of these rats, 100 died or were killed between 8 and 55 days after the start of the experiment ("hepatitic" stage), while 120 rats were examined after 70 to 729 days of the ethionine diet, alternating with Checkers ("cirrhotic" stage). Similarly, 40 rats were given continuously for 71 to 337 days the synthetic diet to which 0.3% ethionine was added ("cirrhotic" stage). In addition, 45 rats which had been given the 0.5% ethionine diet were returned to the Checker diet for 10 to 193 days before being killed. From five of these rats cardiac blood was obtained for chemical determination before return to the stock diet. Twenty-five rats given Checkers for a similar period of time served as controls. Finally, 12 animals were splenectomized, and subsequently 3 served as controls, while 9 animals were given the 0.5% ethionine diet for from 25 to 172 days.

Histologic examination of liver, spleen, and lymph nodes included hematoxylin and eosin, Mallory's aniline blue, and Van Gieson elastica stains; silver impregnation of both formalin- and Carnoy-fixed material, and methyl green and pyronin staining, with and without preceding digestion by ribonuclease, of Carnoy-fixed material. Bone marrow smears were studied with Wright's stain. In selected instances, γ-globulin determinations were carried out on the blood serum both by turbidimetric estimation[12] and by zone electrophoresis with subsequent scanning of the bromphenol-stained paper strips.[13]

Results

I. Hepatic Lesions.—The hepatic lesions are described elsewhere,[14,15] and only a brief summary pertinent to this study is given here.

Stage 1: "Centrolobular degeneration." The livers of animals which were fed the ethionine diet for 8 to 20 days showed hepatocellular degeneration and necrosis or hemorrhage in the lobular center. This was accompanied by periportal accumulation of ductular cells, gradually extending into the lobule.

Stage 2: "Diffuse hepatitis." Diffuse hepatocellular degeneration as well as necrosis and regeneration of single liver cells throughout the lobule were associated with a diffuse interstitial-cell reaction, consisting of ductular and inflammatory cells. Eventually cholangiofibrotic nodules (Fig. 1)

Submitted for publication April 30, 1957.

From the Hektoen Institute for Medical Research of the Cook County Hospital and Northwestern University Medical School.

Supported by grant No. C-2030, United States Public Health Service, National Institutes of Health. Present address of Dr. Popper: The Mount Sinai Hospital, New York.

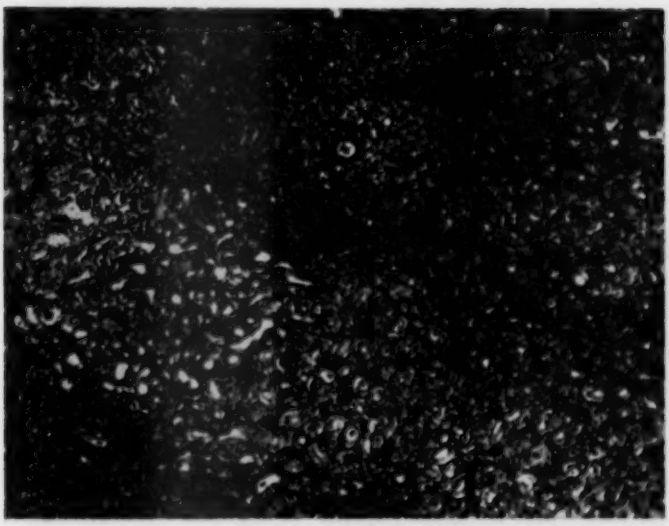


Fig. 1.—Diffuse hepatic damage with single cell necrosis and regeneration. Note excessive amount of cholangiofibrosis. This was associated with splenic pulp fibrosis. Hematoxylin and eosin; reduced ⅙ from mag. × 70.

developed around portal fields, usually in association with liver-cell necrosis. In rats which had received the 0.3% instead of the 0.5% ethionine diet, the hepatocellular changes and interstitial-cell reaction were less conspicuous. Following return of the animals to normal diets, the hepatocellular degeneration and the inflammatory cells gradually disappeared, and the ductular cells became elongated and decreased in number.

Stage 3: "Septal cirrhosis." Along some planes within the lobules argyrophilic fibers aggregated which subsequently became collagenized. This resulted in septa which dissected the lobules into nodules. The latter exhibited little regenerative activity and appeared to exercise little pressure upon the surrounding framework (Fig. 2).

Stage 4: "Coarse nodular cirrhosis." As a result of focally accentuated regeneration,

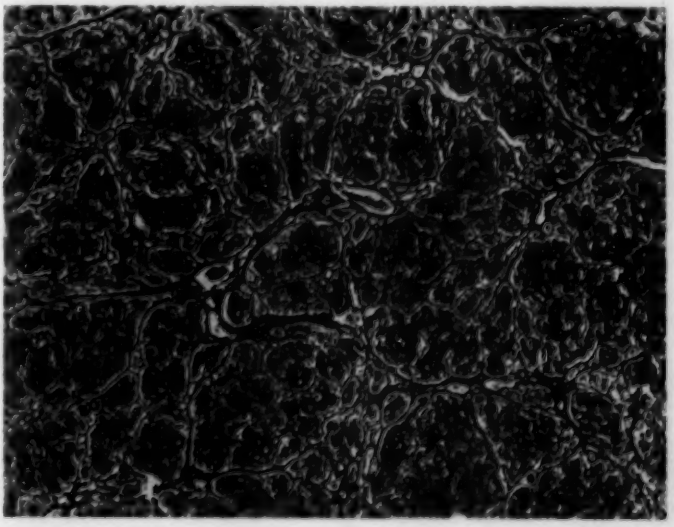


Fig. 2.—"Septal cirrhosis." The parenchyma is dissected by septa, and the nodules exhibit little regenerative activity. Example of animal given ethionine for 55 days and subsequently a normal diet for 5 months. Spleen was normal. Gomori's silver impregnation; reduced ⅙ from mag. × 70.

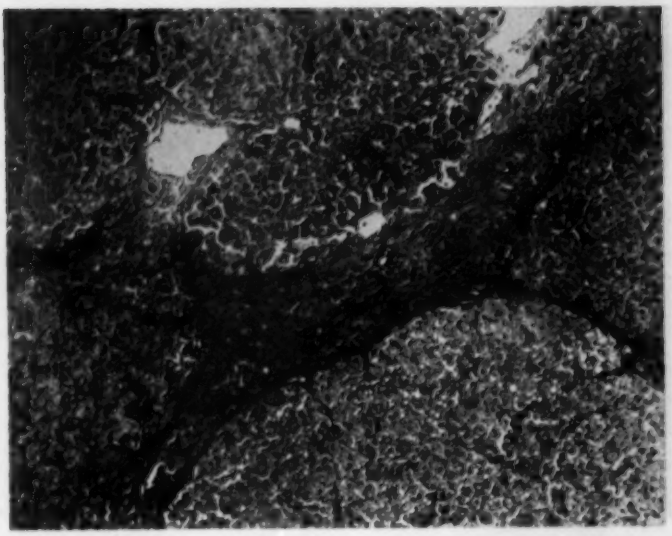


Fig. 3.—"Coarse nodular cirrhosis." Nodules with marked regenerative activity. Note atrophy of intervening parenchyma. Hematoxylin and eosin; reduced ⅙ from mag. × 70.

hyperplastic or regenerative nodules formed, with several cell-thick plates and a poorly outlined reticulum framework. As these nodules enlarged, they appeared to compress the surrounding tissue (Fig. 3). The liver cells disappeared, and the surrounding connective tissue frame-work condensed. No consistent relationship was observed between the cytoplasmic basophilia—as indicated by pyronin stains—of the Kupffer cells and other mesenchymal elements and the serum γ-globulin levels.

II. Splenic Lesions.—1. Normal Spleen: In the spleen of the control rats, the white pulp was prominent and surrounded by a wide mantle of large cells with pale-staining nuclei. This mantle represented the bulk of the red pulp (Fig. 4), which contained otherwise only a few scattered groups of small mononuclear cells intermixed with

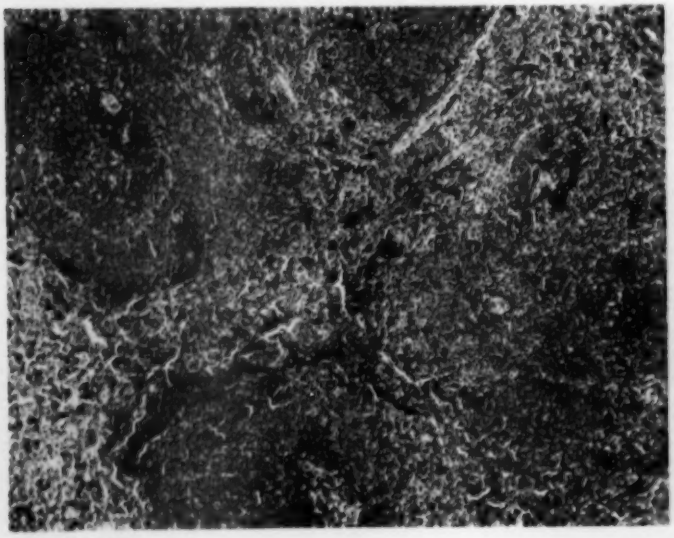


Fig. 4.—Normal spatial arrangement of the Malpighian follicles and their mantles. This spleen is from an animal with advanced cirrhosis returned to a normal diet. Note the pulp fibrosis in the center. The spleen was of normal size. Hematoxylin and eosin; reduced ⅙ from mag. × 70.

Fig. 5.—Widening of red pulp due to congestion, cellular infiltration, and extramedullary hemopoiesis. Hematoxylin and eosin; reduced ⅙ from mag. × 105.

larger cells. Some of these cells exhibited cytoplasmic pyroninophilia before digestion with ribonuclease.

2. Changes in Ethionine Intoxication: In the various stages of ethionine intoxication, the following features were observed.

(*a*) Cellular infiltration and extramedullary hemopoiesis. In the rat spleen, diffuse and variable congestion; foci of hemopoiesis, and infiltration by segmented leukocytes, mononuclear cells, and plasma cells were frequently encountered. The outer border of the perifollicular mantle became less distinct, and the red pulp widened (Fig. 5). These lesions were invariably present during the early hepatic alterations (Stage 1) and were associated with an increase of the splenic weight to approximately twice the normal (Fig. 6). The alterations were considered transient reactions to sometimes minor insults, since they were noted in control animals which died spontaneously; in the later stages of the ethionine intoxication they were not regularly seen.

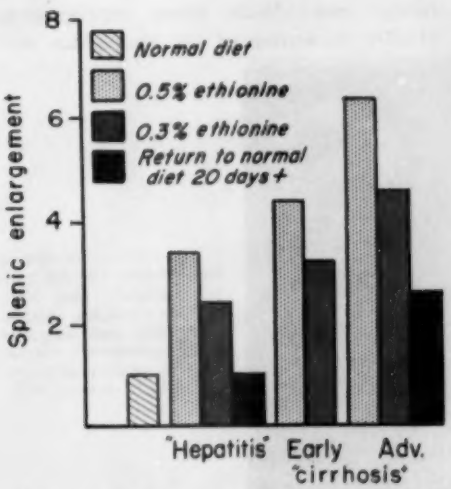


Fig. 6.—Average splenic enlargement in the various hepatic stages in animals given 0.5% and 0.3% ethionine and after return to normal diets for 20 days or longer.

(*b*) Pulp-cell hyperplasia. From the beginning of the "hepatitic" stage throughout the course of the intoxication, a gradual and consistent increase in the splenic pulp cells occurred. These were observed as clusters of large cells with pyronin-staining cytoplasm and low content of nuclear chromatin often showing clumping along the nuclear periphery, and smaller cells with pyknotic nuclei and pyronin-negative cytoplasm (Fig. 7). These cellular foci were frequently accompanied by small mononuclear cells, including, in some instances, plasma cells. The foci first developed in the subcapsular, peri-

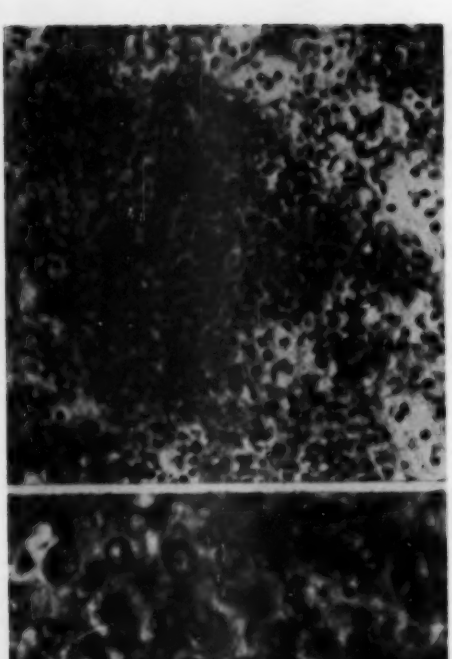


Fig. 7.—Cluster of splenic pulp cells. The cells with poor chromatin content show a bright red cytoplasm with the methyl green and pyronin stain. They do not resemble mature plasma cells, although some suggest cart-wheel arrangement. Note also the darker nuclei; their cytoplasm is pyronine negative. Methyl green and pyronin stain; reduced ⅙ from mags. × 285 and × 490.

vascular, and peritrabecular zones and gradually caused an expansion of the red pulp, with wide separation of the Malpighian follicles and their mantles (Fig. 8). Eventually the pulp-cell hyperplasia was the most prominent feature and accounted for the increase in size up to 10 times the normal (Fig. 6) and an increase in the total pyroninophilic mass. Pulp-cell hyperplasia and splenic weight correlated well with the number of days the animals had been given the diet (Fig. 9); the spleens in animals treated with 0.3% ethionine were significantly smaller than those treated with 0.5% ethionine in the corresponding hepatic stages (Fig. 6). When the activity of the hepatic process was reduced by substitution with normal diets, the pulp cells and splenic weights decreased (Fig. 6) in spite of the persistence of septal or coarse nodular cirrhosis.

(*c*) Follicular and perifollicular changes. Occasional hemorrhage around the central artery of the white pulp or around the smaller perifollicular vessels was encountered, more often in animals with cirrhosis than in those with hepatitis, and was not seen in animals returned to normal diets. Perifollicular necrosis or fibrosis was only rarely seen. With silver impregnation, fibrillar thickening of the Malpighian cor-

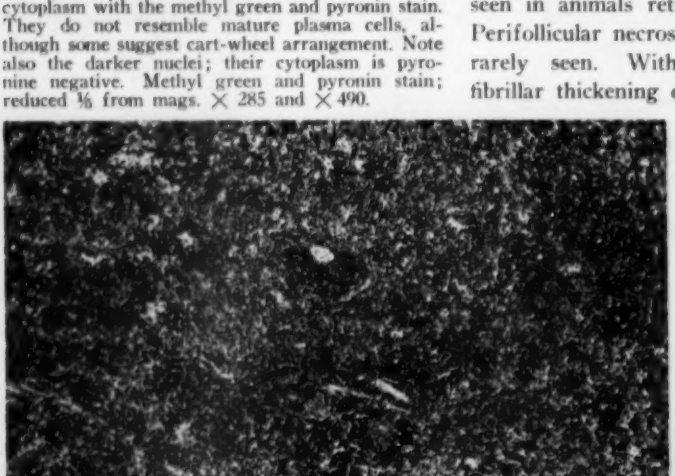


Fig. 8. — Pulp-cell hyperplasia. The red pulp is enlarged; the Malpighian follicles, widely separated, and the pulp cells, prominent. Hematoxylin and eosin; reduced ⅙ from mag. × 70

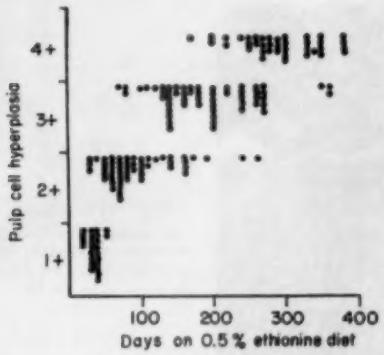


Fig. 9.—Relationship of splenic pulp-cell hyperplasia to number of days on ethionine diet.

puscles (Fig. 10) was noted in 30% of animals with cirrhosis and 22% of animals with precirrhotic lesions. The follicular fibrosis did not as a rule extend beyond the limits of the white pulp and apparently developed independently from the fibrosis of the red pulp.

(*d*) Venous congestion and fibrosis of the red pulp. Towards the later part of the second stage, marked engorgement was noted of the trabecular veins, pulp veins, and sinuses around the trabeculae. As the lesion progressed the sinusoidal engorgement became diffuse and then seemed to contribute to the splenic enlargement. Venous engorgement occurred with equal frequency in animals fed 0.3% and 0.5% ethionine. In the later stages, particularly when coarse nodular cirrhosis was present, prominent vascular spaces appeared about trabecular and pulp veins (Fig. 11). These were bounded by thickened reticulum fibers, which gradually acquired the staining reactions of collagen. In advanced cases these areas often revealed spindle-shaped nuclei with the characteristics of fibroblasts; however, these cells were not conspicuous during the development of the lesion. The described foci of fibrosis did not extend into the perifollicular mantel. Even when advanced they only accounted for a small portion of the splenic enlargement. Of 41 cases of pulp fibrosis encountered, 32 were observed in advanced cirrhosis; 6, in developing cirrhosis, and 3, in the "hepatitic" stage, in the latter usually associated with excessive cholangiofibrosis (Fig. 1). Pulp fibrosis was not seen in six animals with arrested "septal" cirrhosis (Fig. 2). No correlation was demonstrable between the incidence of pulp fibrosis in advanced cirrhosis (ethionine feeding of approximately 150 days or more) and the duration of the ethionine intoxication (Fig. 12).

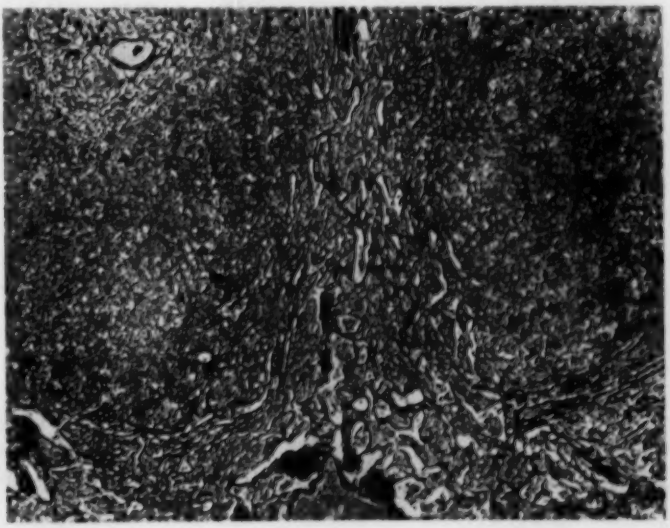


Fig. 10.—Fibrosis of Malpighian follicle and red pulp. These two lesions are not linked anatomically. Gomori's silver impregnation; reduced ⅓ from mag. × 70.

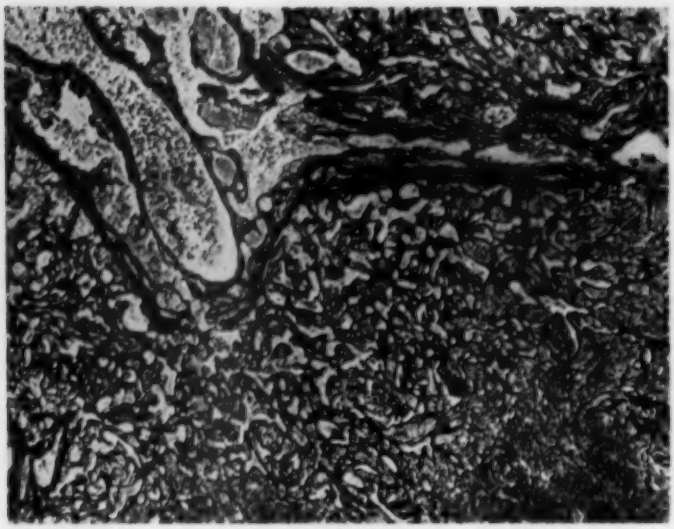


Fig. 11.—Fibrosis of red pulp in perivascular location. Gomori's silver impregnation; reduced ⅓ from mag. × 70.

III. Alterations of Serum γ-Globulin.—The γ-globulin was slightly elevated in the "hepatitic" and markedly so in the "cirrhotic" stage (Table). It was higher in cirrhotic rats which had received 0.5% ethionine than in those which had 0.3% ethionine. The γ-globulin elevation seemed to parallel the degree of pulp-cell hyperplasia (Fig. 13) and the size of the spleen (Fig. 14), which in turn paralleled the "total pyroninophilic mass." After return to normal diets for at least 20 days, despite persistence of cirrhosis, the γ-globulin values decreased parallel with pulp-cell hyperplasia and pyroninophilia.

Splenectomy did not alter the γ-globulin levels and did not prevent its elevation in cirrhotic animals; if anything, the levels were higher than in animals without splenectomy (Table).

IV. Changes in Bone Marrow and Lymph Nodes.—The bone marrow smears failed to reveal an increase in plasma cells in both "hepatitic" and "cirrhotic" stages before or after splenectomy.

The abdominal lymph nodes were enlarged, more so in the cirrhotic than in the hepatitic stages. This was associated with a parallel increase in cytoplasmic pyroninophilia in the medullary cords, which was particularly conspicuous in cirrhotic animals

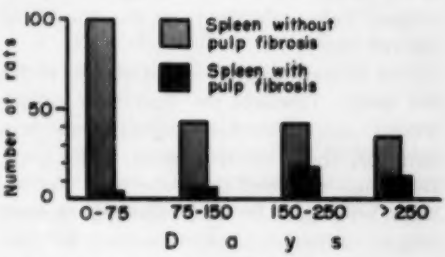


Fig. 12.—Incidence of pulp fibrosis in the various stages of the experiment. Nodular reconstruction is usually complete at approximately 150 days.

after splenectomy (Fig. 15). The nuclear characteristics of the pyronin-staining cells in the spleen and lymph nodes were remarkably similar. As in the spleen, the pyronino-

Mean Values of Serum γ-Globulin Levels

No. of Animals		Serum γ-Globulin, Gm./100 Ml.	
		Turbidimetric	Electrophoretic
Normal	19	0.60	1.10
0.5% Eth. "hepatitis"	10	0.80	1.60
0.5% Eth. "cirrhosis"	19	1.20	1.80
0.3% Eth. "cirrhosis"	5	0.99	1.24
0.5% Eth. "cirrhosis"	5	1.58	...
Same after return to normal diets	5	0.78	...
Splenectomized animals			
Controls	3	0.59	0.66
0.5% Eth. "cirrhosis"	5	1.68	1.73

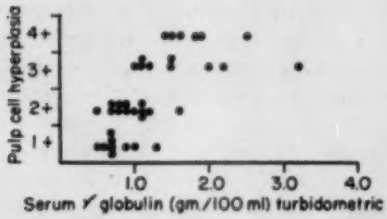


Fig. 13.—Relationship of pulp-cell hyperplasia and γ-globulin levels.

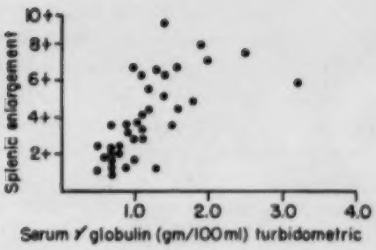


Fig. 14.—Relationship of splenic enlargement and γ-globulin levels.

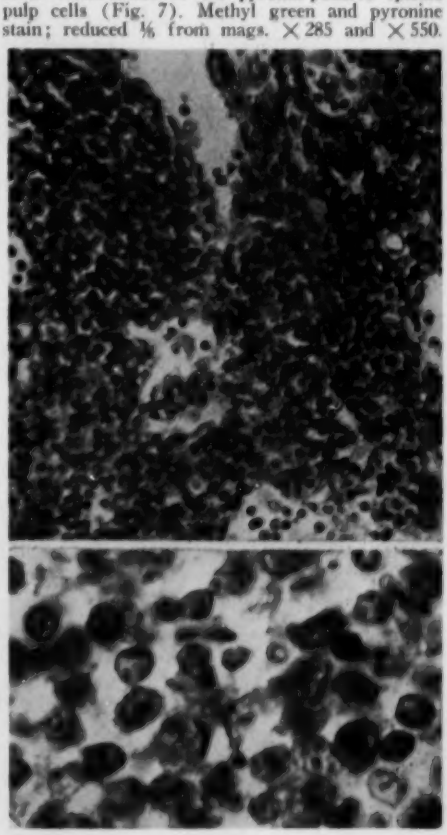
philia paralleled the elevation of the γ-globulin levels.

Comment

The splenic changes observed in ethionine-produced cirrhosis consist of (*a*) pulp-cell hyperplasia, (*b*) venous and sinusoidal engorgement followed by focal pulp fibrosis, (*c*) follicular fibrosis, (*d*) perifollicular hemorrhage or fibrosis, and (*e*) cellular infiltration and extramedullary hemopoiesis. Of these changes, the pulp-cell hyperplasia is the most prominent; it may be observed as an insidious and progressive process long before signs of portal stasis are noticeable and eventually constitutes the bulk of the splenic enlargement up to 10 times its original size. The degree of the splenomegaly correlates with the activity of the hepatocellular process and its duration, while nodular reconstruction per se seems of little significance. Animals treated with 0.3% instead of 0.5% ethionine exhibit a smaller degree of both hepatic injury and splenic pulp-cell hyperplasia with correspondingly smaller spleens. Similarly, after cirrhotic animals are returned to normal diets, despite persistence of the cirrhotic process, pulp-cell hyperplasia and splenic weight decrease progressively, in "septal" cirrhosis to almost normal size and in coarse nodular cirrhosis to an average three times the normal. These findings clearly indicate the importance of nonhydromechanical factors. Pulp-cell hyperplasia has been previously shown to be independent of portal hypertension; it occurs in the "marsupialized" spleen of cirrhotic animals[9] and does not develop in experimental splenic vein ligation.[16]

The engorgement of trabecular and pulp veins and the ensuing fibrosis around these vessels can be considered the result of portal stasis. The fibrosis resembles human fibrocongestive splenomegaly, and the focal na-

Fig. 15.—Medullary cords of abdominal lymph node in splenectomized animal with cirrhosis. Practically all cells exhibit striking pyroninophilia. Note resemblance to the pyronin-positive splenic pulp cells (Fig. 7). Methyl green and pyronine stain; reduced ⅙ from mags. × 285 and × 550.



ture in rats is probably due to the existence of more adequate anastomoses than in the human.[17,18] In contrast to observations reported in the human [19] or in rat cirrhosis induced by other agents,[8] the pulp fibrosis in ethionine cirrhosis does not originate in the perifollicular zones, but first develops about the trabecular veins; even when advanced, it does not usually extend beyond the outer border of the perifollicular mantle. The incidence of the pulp fibrosis is more closely related to the degree of nodular reconstruction than to the duration of the intoxication. This emphasizes the relationship between nodular reconstruction and portal pressure.[20-23]

Since the incidence of perifollicular hemorrhage and fibrosis in ethionine cirrhosis is low, little can be said about their nature. These manifestations have been attributed to both toxic and mechanical factors.[6,7,24,25] While their occurrence in human splenic vein thrombosis [25] has been taken as evidence of a mechanical factor, in experimental cirrhosis they may be observed before the development of portal stasis [8,22,23] suggesting a toxic factor.

The site of formation of the excess serum γ-globulin in hepatic disorders is not established. While some evidence favors a Kupffer cell [26] or hepatocellular [27] origin, implication of the spleen seems especially challenging in human postnecrotic [28] and in ethionine-produced cirrhosis,[11] in view of the considerable splenomegaly and the markedly elevated γ-globulin in these conditions. It is, therefore, significant that in ethionine cirrhosis a good correlation exists between the γ-globulin levels on the one hand and splenic weight, pulp-cell hyperplasia, and pyroninophilia on the other.

Pyronin (Y) stains, when controlled by ribonuclease, appear specific for ribonucleic acid,[29] which in turn is probably related to protein synthesis.[30,31] The pyronin-staining elements consist of large immature pulp cells as well as a few smaller mononuclear cells, including mature plasma cells. Though the latter are often abundant, their occurrence is variable and bears more relationship to superimposed processes, such as spontaneous death, than to γ-globulin levels. In contrast, the pulp cells represent the constant and continuously increasing elements which correlate with splenic size and serum protein changes. The nuclear pattern of the pulp cells suggests the lymphoid series and in some instances immature plasma cells; however, their spatial arrangement and incidence reveals no relationship to mature plasma cells.

The concept of a splenic origin of excessive γ-globulin formation in ethionine-fed rats is supported by the observation [32,33] that the spleen may be the sole source of antibody formation in the rat after intravenous injection of antigen. Under these circumstances immune antibody formation is associated with hyperplasia of the splenic red pulp and an increase in pyronin staining of the cells.[34-36] The morphologic characteristics of this reaction resemble the pulp-cell proliferation observed in our experiment.[37] The similarity of the cells involved in ethionine cirrhosis and those which proliferate during antibody formation deserves further attention.

Against the spleen being the sole site of excess γ-globulin formation in ethionine-fed rats speaks the fact that splenectomy does not prevent the γ-globulin elevation. Moreover, pyroninophilic cells resembling those in the spleen occur to a parallel degree in the abdominal lymph nodes. They are abundant in splenectomized rats with active cirrhosis. Such correlation cannot be established for the mesenchymal cells of the liver and for the bone marrow. The latter, although considered by some to be a source of excess globulin,[27,38,39] did not display significant plasmacytosis. It would appear that the spleen and lymph nodes play a major role in the γ-globulin elevation in ethionine cirrhosis and that after splenectomy the lymph nodes assume a compensatory function.

Summary

The progression of diffuse hepatocellular degeneration to coarse nodular cirrhosis in ethionine-treated rats is associated with an enlargement of the spleen to 10 times its original size and an elevation of the serum γ-globulin level.

The splenomegaly is attributable mainly to a pulp-cell hyperplasia, which is independent of portal stasis but is related to the activity and duration of the hepatocellular process. Venous engorgement and focal pulp fibrosis, considered sequences of portal stasis, contribute only a small part to the splenic enlargement; these changes parallel the degree of nodular reconstruction of the liver and are often unrelated to the splenic weight.

The splenic pulp-cell hyperplasia is held at least partly responsible for the serum γ-globulin elevation; it is accompanied by a corresponding increase in cytoplasmic pyroninophilia and parallels the serum γ-globulin levels; it decreases proportionately with serum γ-globulin values upon discontinuation of ethionine administration, although hepatic reconstruction persists.

Elevated γ-globulin levels can be related to the pyroninophilia of the abdominal lymph nodes, besides that of the spleen. In splenectomized animals with active cirrhosis the γ-globulin elevation is not prevented but is accompanied by a prominent increase in lymph-node pyroninophilia. This suggests a synergistic role of the spleen and lymph nodes in the serum γ-globulin elevation and a compensatory effect of the lymph nodes in the absence of the spleen.

Cook County Hospital, 1825 W. Harrison St. (12).

REFERENCES

1. Thompson, W. B.: The Pathogenesis of Banti's Disease, Ann. Int. Med. 14:255, 1940.

2. Rousselot, L. M.: The Rôle of Congestion (Portal Hypertension) in So-Called Banti's Syndrome: A Clinical and Pathologic Study of 31 Cases with the Late Results Following Splenectomy, J. A. M. A. 107:1788, 1936.

3. Whipple, A. O.: The Problem of Portal Hypertension in Relation to Hepatosplenopathies, Ann. Surg. 122:449, 1945.

4. Moschcowitz, E.: The Pathogenesis of Splenomegaly in Hypertension of the Portal Circulation: "Congestive Splenomegaly," Medicine 27: 187, 1948.

5. McNee, J. W.: The Spleen, Tr. M. Soc. London 54:185, 1931.

6. McNee, J. W.: Liver and Spleen: Their Clinical and Pathological Association, Brit. M. J. 1:111, 1932.

7. McMichael, J.: The Pathology of Hepatolienal Fibrosis, J. Path. & Bact. 39:481, 1934.

8. Menon, T. B.: The Splenic Reaction in Experimental Cirrhosis and in Pre-Cirrhotic Intoxication, J. Path. & Bact. 46:521, 1938.

9. Cameron, G. R., and de Saram, G. S. W.: A Method for Permanently Dissociating the Spleen from the Portal Circulation (the "Marsupialised" Spleen) and Its Use in the Study of Experimental Liver Cirrhosis, J. Path. & Bact. 48:41, 1939.

10. Himsworth, H. P.: Lectures on the Liver and Its Diseases, London, Oxford University Press, 1947, p. 14.

11. Popper, H.; Dubin, A.; Kushner, D. S.; Kent, G.; Bruce, C., and Herzog, E.: Serum Protein Changes in Postnecrotic Cirrhosis Produced by Ethionine, Fed. Proc. 15:528, 1956.

12. de la Huerga, J., and Popper, H.: Estimation of Serum Gamma Globulin Concentration by Turbidimetry, J. Lab. & Clin. Med. 35:459, 1950.

13. Block, R. J.; Durrum, E. L., and Zweig, G.: Manual of Paper Chromatography and Paper Electrophoresis, Ed. 2, New York, Academic Press, Inc., 1955, p. 407.

14. Popper, H.; de la Huerga, J., and Koch-Weser, D.: Hepatic Injury Due to Conditioned Sulfo Amino Acid Deficiency, Ann. New York Acad. Sc. 57:936, 1954.

15. Popper, H.; Kent, G., and Bruce, C.: Hepatic Lesions Induced by Ethionine, to be published.

16. Menon, T. B.: Venous Splenomegaly: A Study in Experimental Portal Congestion, J. Path. & Bact. 46:357, 1938.

17. Rousselot, L. M., and Thompson, W. P.: Experimental Production of Congestive Splenomegaly, Proc. Soc. Exper. Biol. & Med. 40:705, 1939.

18. Volwiler, W.; Grindlay, J. H., and Bollman, J. L.: Chronic Portal Vein Obstruction in Dogs from Silica Cirrhosis, Gastroenterology 24:405, 1953.

19. Banti, G.: Über Morbus Banti, Folia haemat. 10:33, 1910.

20. Baggenstoss, A. H.: The Significance of Nodular Regeneration in Cirrhosis of the Liver, Editorials, Am. J. Clin. Path. 25:936, 1955.

21. Popper, H., and Schaffner, F.: Liver: Structure and Function, New York, The Blakiston Company, 1957, p. 284.

22. Daniel, P. M.; Prichard, M. M. L., and Reynell, P. C.: The Portal Circulation in Experimental Cirrhosis of the Liver, J. Path. & Bact. 64:53, 1952.

23. Daniel, P. M.; Prichard, M. M. L., and Reynell, P. C.: The Portal Circulation in Rats with Liver-Cell Damage, J. Path. & Bact. 64:61, 1952.

24. Marshall, A. H. E.: Outline of the Cytology and Pathology of the Reticular Tissue, Springfield, Ill., Charles C Thomas, Publisher, 1956, p. 146.

25. Klemperer, P.: The Spleen, in Handbook of Hematology, edited by H. Downey, New York, Paul B. Hoeber, Inc., 1938, Vol. 3, p. 1709.

26. Popper, H.; Bean, W. B.; de la Huerga, J.; Franklin, M.; Tsumagari, Y.; Routh, J. J., and Steigmann, F.: Electrophoretic Serum Protein Fractions in Hepatobiliary Disease, Gastroenterology 17:138, 1951.

27. Schaffner, F.; Turner, G. C.; Eshbaugh, D. E.; Buckingham, W. B., and Popper, H.: Hypergammaglobulinemia in Pulmonary Tuberculosis, A. M. A. Arch. Int. Med. 92:490, 1953.

28. Kunkel, H. G., and Labby, D. H.: Chronic Liver Disease Following Infectious Hepatitis: II. Cirrhosis of the Liver, Ann. Int. Med. 32:433, 1950.

29. Taft, E. G.: The Specificity of the Methyl-Green Pyronin Stain for Nucleic Acids, Exper. Cell Res. 2:312, 1951.

30. Thorell, B.: Studies on the Formation of Cellular Substances During Blood Cell Production, London, Henry Kimpton, 1947.

31. Caspersson, T.: Cell Growth and Cell Function: A Cytochemical Study, New York, W. W. Norton & Company, Inc., 1950.

32. Rowley, D. A.: The Effect of Splenectomy on the Formation of Circulating Antibody in the Adult Male Albino Rat, J. Immunol. 64:289, 1950.

33. Wissler, R. W.; Robson, M. J.; Fitch, F. W.; Nelson, W., and Jacobson, L. O.: The Effects of Spleen Shielding and Subsequent Splenectomy upon Antibody Formation in Rats Receiving Total-Body X-Irradiation, J. Immunol. 70:379, 1953.

34. Fitch, F. W.; Barker, P.; Soules, K. H., and Wissler, R. W.: A Study of Antigen Localization and Degradation and the Histologic Reaction in the Spleen of Normal, X-Irradiated, and Spleen-Shielded Rats, J. Lab. & Clin. Med. 42:598, 1953.

35. La Via, M. F.; Barker, P. A., and Wissler, R. W.: A Study of the Correlation of Antigen Phagocytosis and the Splenic Histologic Reaction with Antibody Formation in Protein-Depleted Rats, J. Lab. & Clin. Med. 48:237, 1956.

36. Wissler, R. W.; Frazier, L. F.; Soules, K. H.; Barker, P., and Bristow, E. C., III: The Acute Effects of Beta$_2$ Thienylalanine in the Adult Male Albino Rat: Observations on Nitrogen Balance, Antibody Formation, and Tumor Growth, A. M. A. Arch. Path. 62:62, 1956.

37. Wissler, R. W.: Personal communication to the authors.

38. Fagraeus, A.: Antibody Production in Relation to the Development of Plasma Cells: In Vivo and in Vitro Experiments, Acta med. scandinav. (Supp. 204) 130:3, 1948.

39. Björneboe, M., and Sormsen, H.: Experimental Studies on the Role of Plasma Cells as Antibody Producers, Acta path. et microbiol. scandinav. 20:649, 1943.

Morphologic Features of an African Strain of Histoplasma in Hamsters and Mice

JAN SCHWARZ, M.D., Cincinnati, and EDOUARD DROUHET, M.D., Paris

Large tissue forms of Histoplasma capsulatum have been repeatedly reported, especially from Africa,[1,7] but Vanbreuseghem suggested a distinct species, which he named Histoplasma duboisii, in 1952.[6] We have investigated the increment of large tissue forms of Histoplasma and have established that forms up to 20μ could be obtained in tissue explants from American strains.[9] We also have demonstrated large forms to be present in culture tissue phase in 11 of 13 American strains with considerable variation in the number of large yeast cells related to the individual strains, the age of the culture, the type of medium employed, etc.[4,5]

Method and Material

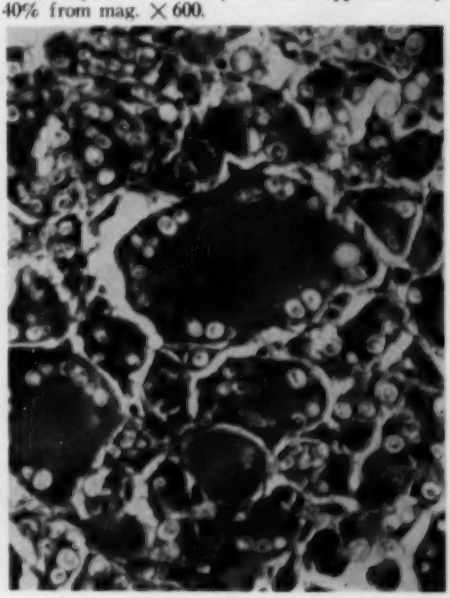
One of the characteristics of the African strain isolated by Vanbreuseghem was the relative lack of pathogenicity for animals. In the present experiment, therefore, 168,000,000 small and 350,000 large yeast cells of African Strain 44 were injected intraperitoneally into eight male hamsters and five male mice. The animals were kept for 4 to 67 days. The hamsters were killed after 4 (H1), 6 (H2), 9 (H3), 13 (H4), 16 (H5) and 67 days (H6, H7, H8), respectively. All five mice were killed after 67 days (M1 through M5).

Results

At autopsy animals H6, H7, and H8 had very large livers and spleens; fibrinous exudate was covering the peritoneum and the spleen. The spleens had a very peculiar whitish-pink color, resembling total necrosis of the organ.

The development in hamsters is microscopically as follows: first (H1) only the peritoneum is involved, with numerous epithelioid cell tubercles and numerous multinucleated giant cells. In the center of some tubercles there are polymorphonuclear leukocytes. The organisms vary greatly in size (from 2μ-10μ); there are a few large but many small forms. The large forms have thin walls and are always associated with groups of small cells. Most large

Fig. 1.—Enormous giant cells containing large yeast cells have invaded the peritoneum and replaced the other cells; notice the large uniformly round to ovoid yeast cells within the giant cells, often surrounded by a "halo." Hamster 67 days after injection of African Histoplasma strain 44. Hematoxylin and eosin; reduced approximately 40% from mag. × 600.



Submitted for publication April 15, 1957.

This study was in part supported by a grant (E-576) from the National Institutes of Health, U. S. Public Health Service.

From the Clinical Laboratories, Jewish Hospital, and the Laboratory of Mycology, Cincinnati General Hospital (Dr. Schwarz); from the Service de mycologie et de physiologie végétale, Institut Pasteur (Dr. Drouhet).

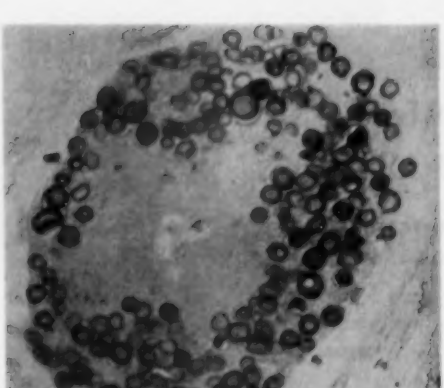


Fig. 2.—Very numerous and large yeast cells within gigantic giant cell, the outlines of which are only faintly visible. Notice considerable variation in cell size, with majority of cells larger than 10μ. The two largest cells (left center and upper-field center) show unmistakable budding. Liver hamster 67 days after infection. Gridley stain; reduced approximately 40% from mag. × 600.

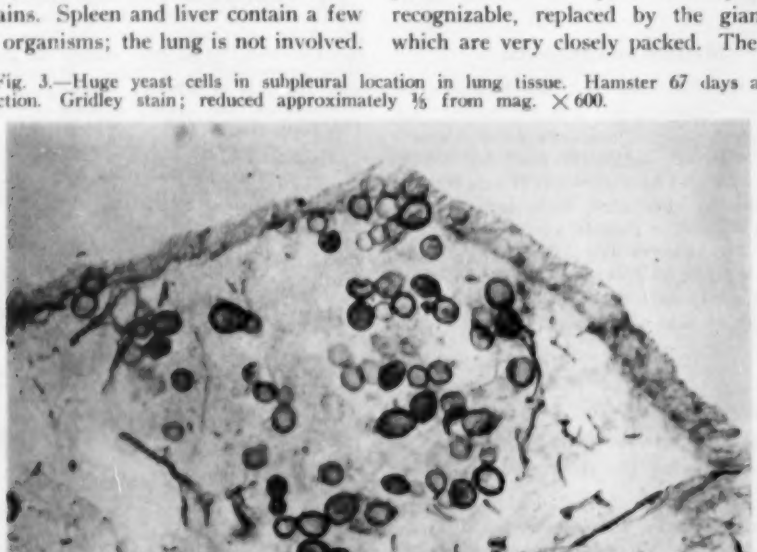
forms are ovoid; few are round. There is no multiple layer formation ("onion" phenomenon), as we have seen in several American strains. Spleen and liver contain a few isolated organisms; the lung is not involved.

Two days later (H2) tubercles are seen in liver, lung, and spleen, in addition to those seen in the peritoneum. Huge giant cells have developed in the peritoneum, which soon became the dominating feature. Large forms of yeast are prominent in the giant cells. The proportion of large to small cells is 40:60. Without exception the large forms have thin walls.

The tubercles in the organs become more and more numerous, and the large forms become more prominent numerically. In the spleen (H4) some of the large forms seem to have two walls, but close inspection shows that the internal nodular appearance probably represents the lipoid bubble. There is definitely no "onion-layer" phenomenon.

The animals which lived two months show fantastic lesions. The peritoneum is transformed into a mass of giant cells, some of which measure 210μ in the greatest diameter. These giant cells as a rule have peripherally located large forms of Histoplasma, which have a wide halo. There is no "onion" phenomenon. The spleen is completely unrecognizable, replaced by the giant cells, which are very closely packed. The lesions

Fig. 3.—Huge yeast cells in subpleural location in lung tissue. Hamster 67 days after injection. Gridley stain; reduced approximately ⅓ from mag. × 600.



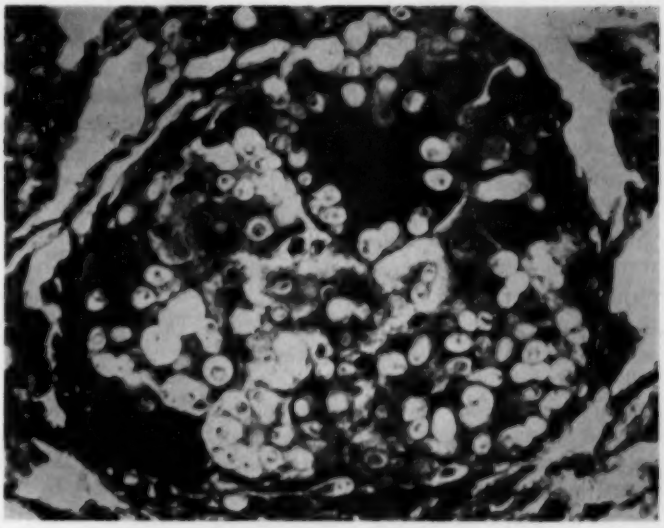
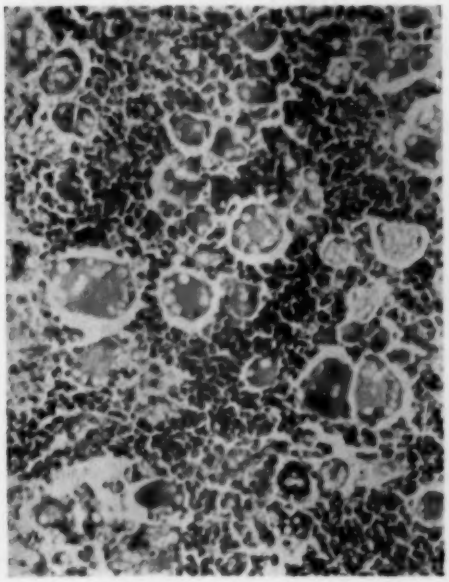


Fig. 4.—Large giant cells and apparently free yeast cells accumulated in nodule in interstitium of lung. Hamster 67 days after infection. Hematoxylin and eosin; reduced approximately ⅓ from mag. × 600.

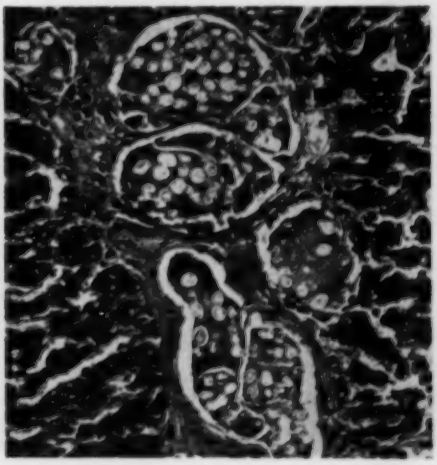
in the three animals (H6, H7, H8) are practically identical. The involvement is seen in lung, liver, spleen, adrenals, kidney, and submucosa of bowel, a truly disseminated disease.

Fig. 5.—Numerous giant cells, with large yeast cells and lymphocytes in infiltrate surrounding the kidney (perirenal fat capsule). Hamster 67 days after infection. Hematoxylin and eosin; reduced approximately 40% from mag. × 300.



The five mice which were killed after 67 days showed lesions very similar to the ones observed in the hamsters, which were likewise killed 67 days after infection. All organs contained large yeast cells of an average diameter of 10μ-12μ, with occasional forms measuring 14μ and, exceptionally, of 22μ. Small yeast cells of 2μ-4μ were also visible.

Fig. 6.—Huge giant cells filled with large yeast cells of African Histoplasma in interstitium of liver. Hamster 67 days after injection. Hematoxylin and eosin; reduced ⅓ from mag. × 600.

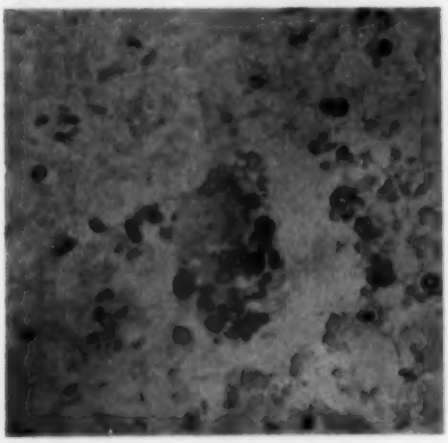


Comment

This study, undertaken to learn whether the African strain H. duboisii can become lethal to animals, proved in the first place that no mortality occurred even with such large doses as 168,000,000 organisms per animal. Secondly, it provided us with material for microscopic study. In previous work we have noted that peculiar large forms with concentric rings are formed by many American strains in the hamster, but these starch-like, or "onion"-layered, forms can be easily differentiated from the huge cells seen in the present experiment. In the latter, "onion forms" hardly ever occurred. On the contrary, most cells were round or only slightly ovoid and had thick walls, which were rather refractile in hematoxylin and eosin preparations and which stained uniformly red on Gridley procedure. The identical organisms were observed in enormous numbers in mice, which is contrary to the reaction of this animal species to the injection with American strains of H. capsulatum.

We therefore can point out that in the animal experiment the strain named H. duboisii by Vanbreuseghem shows some differences from the morphologic appearance of the American strains of H. capsulatum.

Fig. 7.—Large and small cells in peritoneum of mouse 67 days after injection of African Histoplasma. Gridley stain; reduced ⅓ from mag. × 500.



These differences refer to both the morphology of the yeast cell in tissues and the tissue reaction provoked by the organisms in mice and hamsters.

Neither hamsters nor mice died from intraperitoneal injection of a culture suspension containing 168,000,000 small and 350,000 large yeast cells of Strain 44. But this lack of virulence is not a definite characteristic for strains from African Histoplasma. In a preliminary study, one of us [3] mentioned two recent strains which were isolated from African Histoplasma with large cells and which were lethal to hamsters and mice on intraperitoneal injection. A similar experience was recently described by Depoux and Merveille.[2] On the other hand, an African strain isolated from monkeys imported from French Africa showed no virulence.[5] It may be pointed out that only 1 of 13 American strains which were tested by us had not been lethal for hamsters, but only 20,000,000-30,000,000 cells were injected in this experiment.

In the average American strain of H. capsulatum large cells are hardly ever seen with a simple thick wall. Instead, the repeatedly mentioned onion-like structures are seen. Large forms in the mouse are rare. In contrast, the African Strain 44 produced innumerable large cells with a distinct and thick wall in hamsters and in mice.

The American strains produce very violent peritonitis and subsequent histiocytic and leukocytic tissue reaction, especially in the hamster. Strain 44 runs through a phase of true inflammatory response in the peritoneum and organs, but after two months one sees almost exclusively large (210μ) and, in our opinion, unique multinucleated giant cells, densely filled with the above-described large and thick-walled yeast cells.

We have the definite impression that in the American strains the small yeast cell becomes surrounded by a capsule-like material, at present unidentified, which is almost exclusively provided by hamster tissues. On the contrary, Strain 44 shows large yeast cells of 10μ-12μ (seldom up to

22μ). These large yeast cells of Strain 44 appear to be morphologically more similar to the giant forms observed by one of us in tissue explants [9] and represent true enlargement of yeast cells, due either to inhibition of budding or to some other mechanism.

Summary

One hundred sixty-eight million yeast cells of the African strain named Histoplasma duboisii by Vanbreuseghem did not produce death in eight hamsters and five mice kept up to 67 days. Large yeast cells (average size 10μ-12μ) were numerous in the organs of the killed animals, and unique giant cells engulfed the organisms. These findings indicate that this strain can be differentiated in vivo experimentally from the American strains of H. capsulatum.

It will be necessary to accumulate many more African strains in order to get a definite idea as to whether the strains which produce large cells in vivo represent a variety or a subspecies of H. capsulatum or whether they are a separate species.

The Jewish Hospital Association (29).

REFERENCES

1. Catanei, A., and Kervran, P.: Nouvelle Mycose humaine observée au Soudan français, Arch. Inst. Pasteur Algérie 23:169-172, 1945.

2. Depoux, R., and Merveille, P.: L'Histoplasmose en A. E. F., Semaine hôp. Paris, to be published.

3. Drouhet, E.: Quelques Aspects biologique et mycologique de l'histoplasmose, Semaine hôp. Paris, to be published.

4. Drouhet, E., and Schwarz, J.: Croissance et morphogenèse d'Histoplasma: I. Étude comparative des phases mycélienne et levure de 18 souches d'Histoplasma capsulatum d'origine américaine et africaine, Ann. Inst. Pasteur 90:144-160, 1956.

5. Drouhet, E., and Schwarz, J.: Comparative Studies with 18 Strains of Histoplasma: Morphology in Tissues and Virulence of African and American Strains. J. Lab. & Clin. Med. 47:128-139, 1956.

6. Dubois, A.; Janssens, P. G.; Brutsaert, P., and Vanbreuseghem, R.: Un Cas d'histoplasmose africaine: Avec Une Note mycologique sur Histoplasma duboisii n. s. p., Ann. Soc. belge méd. trop. 32:569-583, 1952.

7. Duncan, J. T.: A Unique Form of Histoplasma, Tr. Roy. Soc. Trop. Med. & Hyg. 40:364-365, 1947.

8. Mariat, F., and Segretain, G.: Étude mycologique d'une histoplasmose spontanée du singe africain, Cynocephalus babuin, Ann. Inst. Pasteur 91:874-891, 1956.

9. Schwarz, J.: Giant Forms of Histoplasma Capsulatum in Tissue Explants, Am. J. Clin. Path. 23:898-903, 1953.

Disseminated Lupus Erythematosus

Histopathology, Morphogenesis, and Relation to Allergy

GUNNAR TEILUM, M.D., and HEMMING E. POULSEN, M.D., Copenhagen

The interpretation of the various histological changes in disseminated lupus erythematosus (D. L. E.) has long been the subject for discussion. The most important theories and papers on the subject up to 1942 have been reviewed by Libman.[1] Klemperer, Pollack, and Baehr[2] believed that the basic pathomorphologic feature was a widespread damage of collagen, and the term "collagen disease" was used to collect descriptively into a group such maladies as were characterized morphologically by systemic alterations of the intermediate substances of the connective tissue, especially of the collagen fibers. In discussing the rationale of the term and of the concept, Klemperer stated that it did not imply a pathogenetic definition; in fact the idea was proposed mainly in order to call attention to the significance of the connective tissues as the site of morbid changes.

The characteristic histological lesions in D. L. E. were later interpreted as a manifestation of hypersensitivity or allergy (Fox,[3] Teilum,[4,5,8] and Rich[6]). The hypothesis of allergy was not based solely upon the presence of a widespread fibrinoid alteration of the connective tissue analogous with Klinge's pathogenetic generalization. Teilum thus described nodular granulomatous and necrotic lesions in serosa, lungs, and lymph nodes, as well as necrotizing panvasculitis with subintimal fibrinoid degeneration, endothelial proliferation, and intimal granulomas, in typical cases of D. L. E. which also displayed the characteristic glomerular lesions of the kidney and the onion-skin lesions of the splenic vessels. In such cases of D. L. E. striking similarities in histological picture, phase development, and cellular reactions were thus found, for instance, to the morphological equivalents in various allergic syndromes described by Bergstrand[7] and others. Nor has the allergic pathogenesis, of course, been the least disproved by the findings of local fibrinoid connective-tissue damage in a variety of conditions where the hypothesis of allergy cannot be seriously entertained, such as in the base of peptic ulcers and in acute bacterial infections.

Further histological studies suggested a primary cellular origin of the characteristic wire-loop lesions in the kidneys, as well as of the onion-skin lesions of the spleen, on the analogy of the local precipitation of other homogeneous substances, such as hyalin, paramyloid, and amyloid.[8] The association of glomerular and periarterial splenic lesions with plasmocytosis and hyperglobulinemia in a number of cases suggested fundamental morphogenetic similarities of such lesions in D. L. E., sarcoidosis, and paramyloidosis, also included in the group of related pararheumatic diseases.[8]

The common primary basis was supposed to be determined by a stimulation of the immune mechanism involving mesenchymal derivative cells of the reticuloendothelial system and the glomeruli of the kidney. It was generally assumed later on that stimulation of the antibody-forming apparatus occurs in D. L. E. (Smith[9]).

The introduction of the lupus erythematosus (L. E.) cell test by Hargraves et al.[10] led to increased recognition of and interest in D. L. E. The specificity of this test is

Submitted for publication April 12, 1957.

From the Laboratory for Rheumatic Research, The University Institute of Pathological Anatomy.

still being discussed. The demonstration of L. E. cells has been considered pathognomonic of D. L. E. On the other hand, L. E. cells have been demonstrated in diseases other than lupus in exceptional instances, including, for example, isolated cases of hemolytic anemia, multiple myeloma, amyloidosis, active chronic viral hepatitis, penicillin sensitivity reactions, and during the treatment of hypertension with hydralazine.

In 1950, the work of Klemperer et al.[11] appeared in which they called attention to the hematoxylin-stained bodies first described by Libman and Sacks, in 1924, and again, in 1932, by Gross.[12] Klemperer's observations took their origin from the histochemical analyses of the bodies, which showed that they contained depolymerized deoxyribose nucleic acid, derived, as previously supposed by Gross, from nuclear chromatin of mesenchymal cells. Many nuclei of leukocytes and histiocytes stained purple of the same tint as the hematoxylin-stained bodies. The nuclei of such cells were found to be homogeneous, with loss of their chromatinic structure, and such nuclear changes were often present in cells around the hematoxylin-stained bodies. The nuclear changes thus consisted in homogenization and purple staining with hematoxylin. A controlled Feulgen reaction stained the bodies brilliant red, and these showed a very great absorption of ultraviolet rays at 2537 A.

The hematoxylin-stained bodies form large aggregates in the endocardial vegetations and lymph nodes and smaller accumulations and single free bodies in the glomeruli; in the walls of vessels in many organs, especially the ovary, and in connective tissue. In D. L. E. alone the free hematoxylin-stained bodies were seen, and agglomerations of these bodies in large masses occur only in this disease. For these reasons, Klemperer et al. (1950) felt justified in concluding that the free hematoxylin-stained bodies are a specific structural criterion of D. L. E. and that they originate in the nuclei of mesenchymal cells. The inclusions of the L. E. cells have been shown to contain depolymerized deoxyribose nucleic acid, similar to the hematoxylin-stained bodies in the tissues of D. L. E.

These observations led Klemperer to assume that the "fibrinoid" substance in D. L. E. is a degraded nucleoprotein and different from the "fibrinoid" substances in other disorders because the metabolic disturbance which is responsible for the occurrence of this type of "fibrinoid" substance is unique for D. L. E. Klemperer adds that the theory of hypersensitivity has been challenged by these morphologic disclosures.

Further studies on lupus material (Gueft and Laufer [13]) revealed the presence of "an admittedly obscure substance capable of coupling with Schiff's reagent after periodic acid oxidation." The hematoxylin-body aggregate in conventional hematoxylin-eosin-stained sections disclosed rather striking variations in the intensity of hematoxylin staining and Feulgen reaction of the bodies. Some aggregates showed a mottling of hematoxylin-eosin staining. Others were even completely eosinophilic and showed no staining in Feulgen preparations. Gueft and Laufer found that the origin of the periodic-acid-Schiff (PAS)-positive substance in the bodies "is difficult to ascertain," as there is no evidence of any PAS-positive material in the normal nuclei, either from in vitro studies or cytochemical analyses, and considered the possibility of incorporated substances from an extracellular source. The tinctorial variations of the hematoxylin bodies from hematoxylin affinity to eosinophilia directed the attention to the eosinophilic material commonly seen in lupus, the "hyaline thrombi" and the "wire loops" of the glomeruli. Gueft and Laufer found many of these to be quite colorless in Feulgen preparations, but an appreciable number had a faintly positive reddish tinge, denoting the presence of deoxyribose nucleic acid (DNA). Karyorrhexis and karyolysis of the usual kind were also seen.

The demonstration by Klemperer and his associates that the hematoxylin-stained bodies in D. L. E. contain depolymerized

DNA derived from nuclear chromatin is most important, primarily because it correlates certain morphologic changes, apparently with a high degree of specificity, with the L. E.-cell phenomenon, which has led in decisive respects to increasing insight in the pathology of this puzzling disease.

However, in contrast with these authors, we do not believe that the degradation of nuclear chromatin represents a primary phenomenon in the pathology of the disease, decisive to the development of "fibrinoid." It can also be questioned whether it is justifiable in general to consider the "fibrinoid" substance in D. L. E. as a type that is unique for this disorder and whether the development of the "fibrinoid" substance and the formation of "wire loops" and "hyaline thrombi" in D. L. E. are initiated by and necessarily related to such degradation of nuclear protein.

Finally, we do not agree that the recent morphologic observations in D. L. E. will challenge in any way the theory of allergy, which during recent years has been strongly supported through the disclosures of the L. E.-cell phenomenon.

It is the object of the present study, first, to review and discuss some of the theories which have been advanced in attempts to correlate morphologic features and pathogenesis and, second, to emphasize certain basic features in the morphogenesis of the lesions in D. L. E. and those occurring in other mesenchymal diseases and experimental conditions.

Material

Histologic material from 15 typical cases of D. L. E. forms the basis of the present study. Specimens of kidney, lung, pleura, and heart were examined in all 15 cases, whereas sections of the spleen were available in 14; of liver and adrenal glands, in 13, and of lymph nodes, in 11 cases.

Sections were stained with hematoxylin and eosin, by the Van Gieson method, and by the Unna-Pappenheim and the periodic acid-Schiff techniques.

Histologic Findings

The lesions observed microscopically in the 15 cases were as follows: granulomatous and nodular necrotic lesions in 5 cases, hematoxylin-stained bodies in 6, glomerular lesions in the kidneys in 14 (including focal glomerulitis in 14, "wire loops" in 12, "hyaline thrombi" in 9, crescents in the capsular space in 3, and small disseminated subcortical necroses in 1 case). The onion-skin lesion of the spleen was present in all the 14 cases examined. Plasmocytosis was found in the spleen in 8 out of 15 cases; in lymph nodes, in 6 out of 11; in kidneys, liver, and myocardium, in one case.

Pathology of Various Lesions in D. L. E.

1. *Granulomas and Nodular Necrotic Lesions in Lungs, Serous Membranes, and Lymph Nodes.*—These were a prominent feature in several cases of D. L. E. The cells within the granulomas were of the nature of epithelioid cells but were often elongated, with less protoplasm, resembling swollen fibroblasts. Studies of sections stained with hematoxylin and eosin showed in these cases no hematoxylin-stained bodies and did not suggest that these granulomas had any relation to a degradation of nuclear protein. But striking accord was found with the morphologic equivalents in periarteritis nodosa and other allergic syndromes (Bergstrand [7]). The pulmonal vessels in two cases presented the picture of a necrotizing panvasculitis associated with granulomatous lesions in the intima (Fig. 1).

The focal nodular necroses, which are common findings in D. L. E. and conditions which are considered manifestations of allergy, such as, for instance, Wegner's allergic granulomatosis, could not be related to primary nuclear changes.

Studies of sections stained with the PAS method of McManus and Hotchkiss showed developmentally a close relation between reticulum cells showing PAS-positive material in cytoplasm and granulomatous and necrotic lesions and suggested that the cytoplasmic PAS-positive substance was the chief source of the necrotic material. Similar transitions from proliferating PAS-

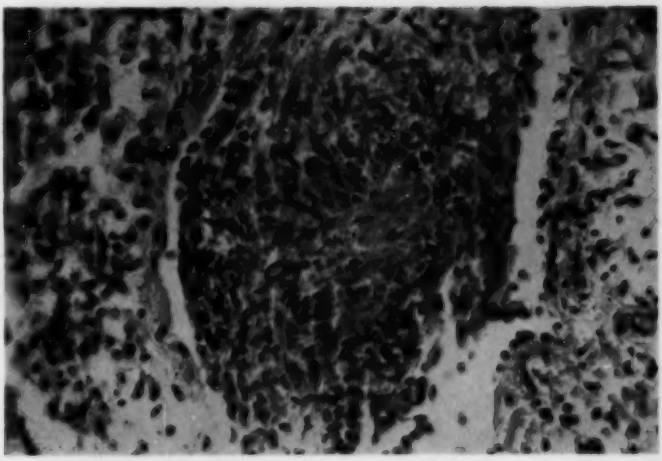


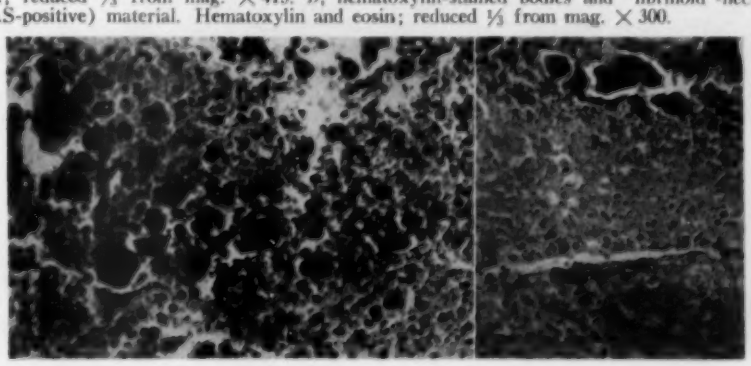
Fig. 1.—Granulomatous lesion in the lung. Van Gieson-Hansen; reduced slightly from mag. × 400.

positive cells to granulomatous and necrotic lesions were a most conspicuous finding in previous experiments in hyperimmunized rabbits.[15]

2. *Hematoxylin-Stained Bodies.*—These were not commonly seen in the present series. They were found in only six cases and generally only in single organs in the same cases. Large aggregates were found in the endocardial vegetations and lymph nodes (Fig. 2), whereas smaller accumulations and single free bodies were observed in the spleen, pancreas, and kidney. The bodies were often surrounded by varying amounts of "fibrinoid material." Histologically it was, however, evident that the fibrinoid material in necrotic lesions was essentially a cytoplasmic PAS-positive product rather than a nuclear product. We admit, however, that the latter change in the cases where it occurs is a most specific criterion in the histological diagnosis of D. L. E.

3. *Glomerular Lesions.*—(*a*) "Wire Loops": With regard to localization and configuration, this change manifests itself as a pronounced local accentuation of capillary wall or basement membrane (Fig. 3). Previous studies in our laboratory [8] showed evidence of a cellular origin of such homogeneous lesions as "wire loops," amyloid, and paramyloid, indicating also morphogenetic similarities; this has also been supported by experimental and histochemical studies on the evolution of glomerular lesions in hyperimmunized rabbits.[14] All transitions were found here from a cytoplasmic product of

Fig. 2.—*A*, aggregates of hematoxylin-stained bodies in lymph node. Hematoxylin and eosin; reduced ⅓ from mag. × 415. *B*, hematoxylin-stained bodies and "fibrinoid"-necrotic (PAS-positive) material. Hematoxylin and eosin; reduced ⅓ from mag. × 300.



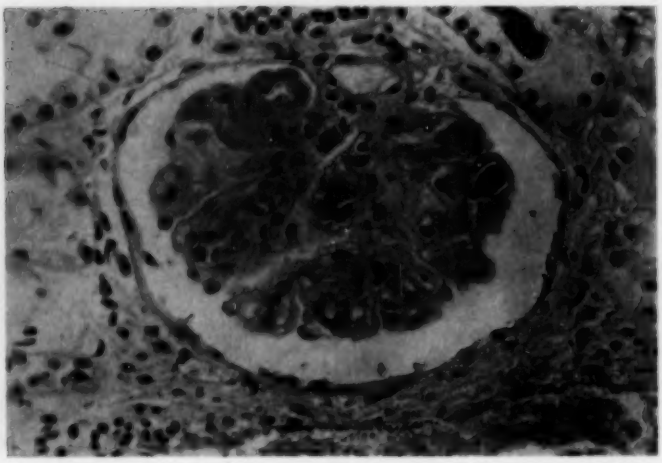


Fig. 3.—"Wire loops" containing homogeneous, eosinophilic, and PAS-positive material. PAS; reduced slightly from mag. ×415.

proliferating endothelial cells of glomeruli to a homogeneous precipitate resembling amyloid or paramyloid in relation to capillary walls or basement membranes in the glomeruli. Such changes are therefore essentially cytoplasmic in origin; this is also indicated by the direct transition from proliferating endothelial cells in the glomerular tufts containing a PAS-positive material in cytoplasm to homogeneous precipitates showing similar tinctorial properties.[15] Adrenocortical hormone of cortisone type was shown to promote this transition to homogeneous precipitate corresponding to the changes in Type 2 nephritis of Ellis and in amyloidosis.[14]

(*b*) "Hyaline Thrombi": The eosinophilic material of the hyaline thrombi (Fig. 4) of the glomeruli in D. L. E. seems to be closely related to the endothelial cellular proliferation and the formation of wire loops. The occurrence of "hyaline thrombi" was found to be most pronounced in the portions of the glomeruli which showed local wire-loop accentuation of the capillary wall. In principle, comparable changes (focal glomerulitis showing transitions to an eosinophilic PAS-positive homogeneous precipitate and "hyaline thrombi") were found in our previous studies on the development of experimental glomerular lesions in rabbits (Fig. 5).

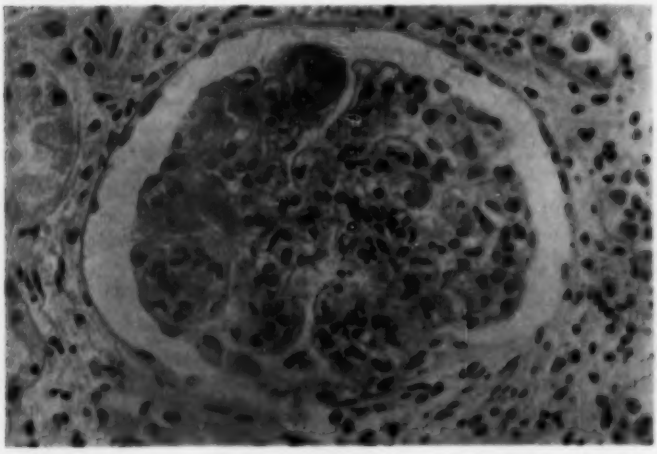


Fig. 4. — Glomerulus showing "hyaline thrombi." PAS; ×415.

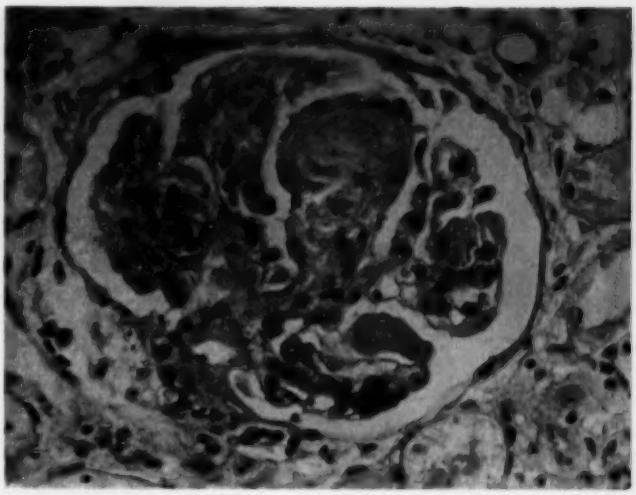


Fig. 5. — Experimental glomerular lesions in rabbit, showing transition from focal glomerulitis to an eosinophilic PAS-positive precipitate and "hyaline thrombi." PAS; × 480.

Our studies on D. L. E. suggested that hyaline thrombi essentially consist of mucoprotein, secreted and liberated by the parent PAS-positive endothelial cells of the glomerulus.

A few glomeruli in the D. L. E. kidney showed coalescence of the eosinophilic cytoplasmic material of the loops and the "hyaline thrombi," so that the glomeruli showed the appearance of a more diffuse change similar to amyloid and paramyloid (Fig. 6).

Gueft and Laufer[13] found that the eosinophilic material in many of the "hyaline thrombi" and "wire loops" of the glomeruli were quite colorless in Feulgen preparations, but an appreciable number had a faintly positive reddish tinge, denoting the presence of DNA. Karyorrhexis and karyolysis of the usual kind were also seen in such glomerular lesions. These workers concluded that DNA is sometimes present in the "wire loops" and "hyaline thrombi."

Because of our own observations, however, we are inclined to interpret the material of these structures as essentially a cytoplasmic product of the glomerular endothelial cells (Fig. 7). This will also explain the PAS-positivity and the morphogenetic features on the analogy of amyloid-like and

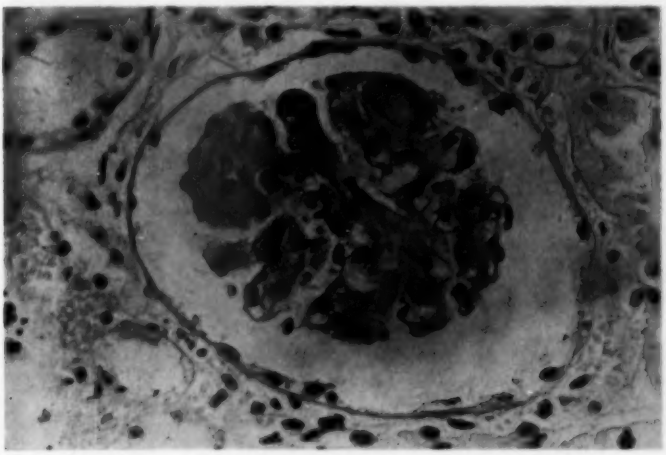
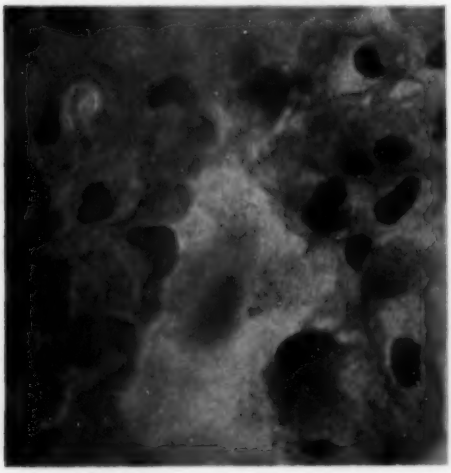


Fig. 6.—Glomerulus in D. L. E., showing coalescence of "hyaline" material of the loops, comparable to amyloid or paramyloid formation. PAS; reduced slightly from mag. × 415.

experimental lesions in hyperimmunized animals.[15]

(*c*) Lupus Nephritis: The recent studies of Muehrcke et al.[16] on the histological and clinical development of lupus nephritis, based upon a total of 61 percutaneous kidney biopsies in 34 patients, represent an important contribution to the histological development of these changes. These workers stated that the earliest detectable histological lesions consisted of minute foci of hypercellularity at the periphery of the glomerular tufts. These lesions were the result of endothelial cell proliferation ("local glomerulitis"). Gradually the hypercellularity became more pronounced, so as to involve the whole glomerulus, and an increasing number of glomeruli became completely involved. An eosinophilic thickening of the glomerular basement membrane was also noted. In some patients the hypercellularity of the tufts was a conspicuous finding, while in others fibrinoid thickening of the basement membrane was more striking and endothelial-cell proliferation was less marked ("local membraneous glomerular nephritis"). "Wire loops" and hematoxylin bodies were not commonly seen in Muehrcke's material.

Fig. 7.—Glomerulus in D. L. E., showing cytoplasmic product of glomerular endothelial cells related to the basement membrane. PAS; ×950.



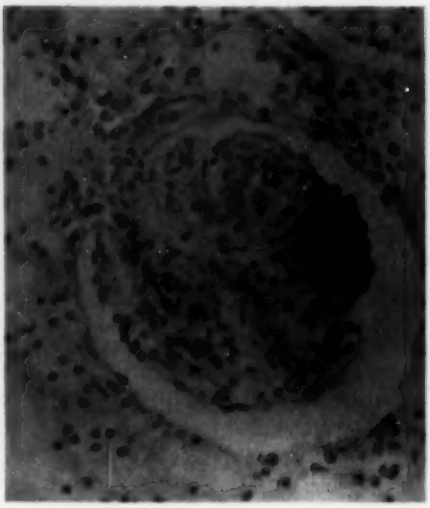


Fig. 8.—Focal glomerulitis (patchy cellularity) in D. L. E. PAS; ×280.

Our own observations of patchy cellularity (Fig. 8), focal membraneous changes, and, in some cases, the picture of a subacute glomerulonephritis with crescent formation (Fig. 9) quite agree with those of Muehrcke et al.; this may lend further support to our view that a primary hypercellularity followed by cytoplasmic transformation accounts for the later membraneous changes in the capillary wall of the glomeruli.

4. *Onion-Skin Lesions of the Spleen.* This lesion was first observed by Sacks (noted in a paper by Libman,[1] and later, by Klemperer. In the paper by Klemperer, Pollack, and Baehr [2] the lesion was said to be present in 19 of the 20 cases studied by them; Kayser [17] found the lesion in 15 out of 18 cases and considered periarterial fibrosis to be present when the periarterial collagen of the follicular and penicillary arteries, which under normal conditions is closely packed and without evidence of hyalinization, was found to present at least three layers around at least half the circumference of the vessels, producing the appearance of concentric rings. This collagen was hyaline in most cases, and in others it consisted partially of granular eosinophilic material. Klemperer considered the lesions specific.

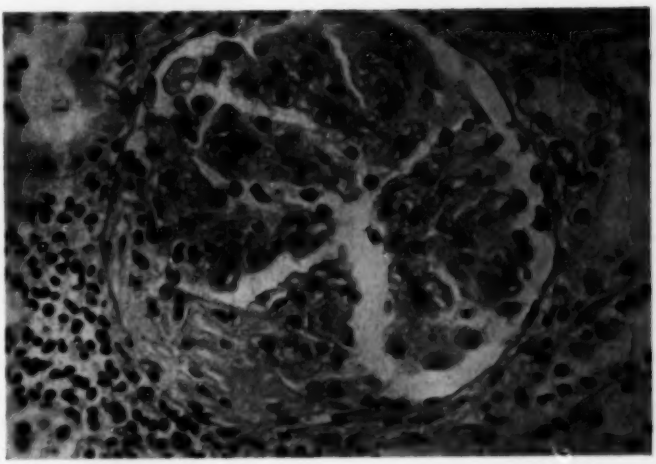


Fig. 9.—Crescent formation in glomerulus. Case of D. L. E., showing the picture of subacute glomerulonephritis. PAS; reduced slightly from mag. × 415.

One of us,[8] who also found this lesion in cases of sarcoidosis and certain other conditions associated with plasmocytosis, related it to a stimulation of the immune mechanism. Plasmocytosis and hyperglobulinemia were characteristic findings in the conditions in which the lesion was found. Morphogenetically it showed similarity to deposition of amyloid and paramyloid in the same site and derived from the same periarterial parent cells. A similar periarterial lesion has been observed in a case of severe hyperglobulinemia associated with affection of the central nervous system.[18] It is of interest that a high percentage of periarterial fibrosis has been found in cases of "thrombocytopenic purpura" (Kaiser). Since either thrombocytopenic or nonthrombocytopenic purpura may be a manifestation of D. L. E., such cases in which the onion-peel lesions were found following splenectomy or at autopsy have been considered cases of D. L. E.[19]

In the present series the onion-skin lesions were found in all the 14 cases in which the spleen was examined microscopically.

5. *Plasmocytosis.*—This was observed in connection with periarterial fibrosis and hyperglobulinemia in various conditions, such as D. L. E., sarcoidosis, and paramy-

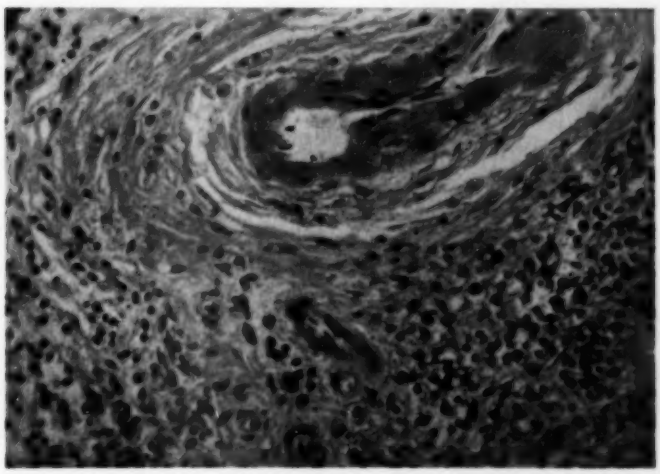


Fig. 10. — Onion-skin lesion and marked plasmocytosis in the spleen (D. L. E). Hematoxylin and eosin; reduced slightly from mag. × 280.

loidosis.[8] Robertson[20] later reported two cases in which sulfonamide drugs were indicated as probably responsible for extensive plasmocytosis and hyperglobulinemia, in addition to the lesions commonly seen in sulfonamide hypersensitivity. Klein and Block[21] studied marrow sections of 60 patients with bone marrow plasmocytosis. The diseases most frequently associated with plasmocytosis in their series were multiple myeloma, rheumatoid arthritis, hepatic cirrhosis, Hodgkin's disease, and granulomatous and collagen disease. Significant elevation of the plasma globulin occurred in 80% of the cases with increased plasma cells.

In the present series of cases of D. L. E., plasmocytosis was found in the spleen and in lymph nodes in about half the number. Pronounced accumulations of plasma cells were often found around the splenic vessels (Fig. 10) and were also conspicuous in a few cases in kidneys and other organs.

Comment

It has been demonstrated recently by Klemperer and his colleagues[11] that the hematoxylin-stained bodies in D. L. E. contain depolymerized DNA derived from nuclear chromatin and that also the eosinophilic material commonly seen in D. L. E. ("wire loops" and "hyaline thrombi") in Feulgen preparations has a faintly positive tinge, denoting the presence of DNA. These findings are of the utmost theoretical and diagnostic importance.

However, when it has been concluded from these and similar findings that the "fibrinoid" substance in D. L. E. in general is a degraded nuclear protein (DNA) (Klemperer) and that the "fibrinoid" formation in systemic lupus is initiated by a peculiar disturbance of DNA metabolism (Gueft and Laufer), it is difficult to accept these interpretations. Nor can we agree with Smith[9] that, to date, there is no convincing explanation of the capillary-loop material in the glomeruli in D. L. E. other than the breakdown of nucleoprotein. Even in hematoxylin bodies Gueft and Laufer[13] found aggregates, and in the capillary loops of the glomeruli in lupus material they found the presence of "an admittedly obscure substance" capable of coupling with Schiff's reagent after periodic acid oxidation; further, many aggregates and loops were found to be completely eosinophilic and quite colorless in Feulgen preparations. These authors found that the origin of the PAS-positive substance "was difficult to ascertain." Obviously it cannot be explained by such primary disturbance of the DNA metabolism.

We want to consider the genesis of this eosinophilic and PAS-positive material in the glomerular loops and hematoxylin body aggregates from quite a different point of view. In discussing the nature of so-called "collagen" and "pararheumatic" diseases, the greatest importance has often been onesidedly attached to abnormalities of the intermediate substances of connective tissue, possibly in conjunction with other abnormalities of the plasma. In a series of previous studies, however, the general significance of a local cytoplasmic precipitation by mesenchymal derivative cells staining with pyronine[8,14] and (or) the PAS technique[15] has been stressed. This type of cytoplasmic transformation disclosed with the PAS technique may account for the local production of amyloid and related lesions. The PAS-positive material demonstrated in cytoplasm of proliferating mesenchymal cells in response to antigens or various unspecific stimuli was considered to be a mucoprotein or glycoprotein produced by the cells, including reticuloendothelial cells and the endothelial cells of the glomeruli. Mesenchymal cellular derivatives, especially reticuloendothelial cells containing a PAS-positive material in cytoplasm, were found to be involved in the morphogenesis of a variety of morphological lesions of the mesenchyme, including amyloid, hyaline, and related changes. These may be associated with alteration in intercellular substances, for instance, in amyloid formation; however, it

was evident that the deposition was initiated and locally determined by the preceding cytoplasmic change in the cells in the same site. The cytochemical findings of hypertrophic strongly positive PAS-positive cells of this category in direct connection with the amyloid material suggested a local precipitation of polysaccharide-containing globulins during the stage of amyloid deposition.[15]

In the cases in which the amyloid formation was the result of a repeated stimulation of the antibody-producing mechanism, a marked plasmocytosis was also found. While also a proportion of the proliferating plasma cells was found to contain PAS-positive material in cytoplasm, it was evident that cells of morphological plasma-cell type did not show a similar cytoplasmic transformation to amyloid.

Therefore, the dysfunctional stage of reticuloendothelial cells, characterized by secretion and local precipitation of PAS-positive material (glycoprotein, mucoprotein) by its cellular components, may explain the essential morphogenetic features of the production of various forms of amyloid or "hyaline" substances, whether or not antigenic substances are involved.

The present studies on lupus material apparently confirm this view of a precipitation of a cytoplasmic product, accounting for the production of the "hyaline" PAS-positive material, such as "wire loops" and "hyaline thrombi," in the glomeruli similar to the findings during amyloid formation.

In this connection it may be mentioned that in experimental animals treatment with cortisone caused a change of the proliferative type of glomerular lesions to membraneous lesions[14] and that cortisone, corticotropin (ACTH), and mechlorethamine hydrochloride (nitrogen mustard) were shown to promote the development of experimental amyloidosis.[22,23]

The assumption that the "hyaline" material of the glomeruli in D. L. E. is a cytoplasmic product also seems compatible with recent studies on the finer structural features of the normal glomerulus, indicating that the basement membrane is primarily a differentiated cytoplasmic product of the endothelial cells (Rinehart et al.[24]).

Probably "fibrinoid" material in D. L. E. and related conditions may include substances of various origin and composition. It is generally supposed that "fibrinoid" is distinct from both collagen and fibrin and that the amorphous ground substance of the connective tissue probably participates in its formation.[25] The homogeneous PAS-positive material of the "wire loops," "hyaline thrombi," and focal necrotic lesions, on the other hand, is apparently essentially related to a cytoplasmic change in proliferating reticuloendothelial cells. We have found no evidence that the "fibrinoid" in D. L. E. is derived from the circulating blood, as has been suggested by Brunson and Davis,[26] who refer to the "collagen diseases" as "systemic fibrinoid diseases."

The finding[27] of aggregates of hematoxylin bodies widely disseminated in many tissues in a case of fulminant allergic angiitis in asthma goes against the supposition of a primary degradation of nuclear protein and lends support to the theory of allergy. We are also unable to agree with Gueft and Laufer[13] in the histogenetic interpretation of similar necrotizing vascular lesions in D. L. E. According to these authors, "The presence [in D. L. E.] of hematoxylin-smudged eosinophilic material within vascular lumina suggests that the fibrinoid alteration of blood vessels is the result of a passage of degraded nucleoprotein circulating in the blood stream into the adjacent vascular wall. The deposited abnormal substance provokes a nonspecific inflammation, with exudation of fine fibrils of fibrin, whereby the eosinophilic homogeneous appearance of the implicated vascular wall is accentuated, with the resulting picture of a necrotizing arteritis." Actually, there is no foundation for such interpretation.

The granulomatous and necrotic vascular and tissue lesions in D. L. E. do not differ morphogenetically from histological lesions

observed, for instance, in various allergic syndromes, Wegener's allergic granulomatosis, or allergic angiitis. The hematoxylin-stained material demonstrable within certain necrotic tissues or glomerular lesions must be considered a special, not primary, alteration. A number of subsequent clinical and serologic observations have also lent support to the hypersensitivity theory. It has been stated that a serum γ-globulin fraction (probably an inactivator of deoxyribonuclease inhibitor) is responsible for the well-known L. E.-cell phenomenon. The L. E. cell itself may be considered the result of an immunophagocytic phenomenon, and hemolytic anemia and thrombocytopenic purpura demonstrated in isolated cases of D. L. E.[28] have been shown in many instances to be secondary to abnormal immunologic reactions.

Summary

The theories which have been advanced in attempts to correlate morphological features and pathogenesis in D. L. E. are discussed.

The assumption that the "fibrinoid" formation in D. L. E. in general is a degraded nuclear protein (DNA) and that the formation of fibrinoid and capillary-loop material in the glomeruli in systemic lupus is initiated by a peculiar disturbance in DNA metabolism could not be confirmed.

Evidence is presented to show that the eosinophilic hyaline material in aggregates and glomerular loops ("wire loops" and "hyaline thrombi"), capable of coupling with the Schiff reagent after periodic acid oxidation, is a cytoplasmic product (mucoprotein or glycoprotein) produced in the same sites by reticuloendothelial cells, i. e., endothelial cells, of the glomeruli.

The University Institute of Pathological Anatomy.

REFERENCES

1. Libman, E.: Some Aspects of "Libman-Sacks Disease," J. Mt. Sinai Hosp. 9:621-629, 1942.

2. Klemperer, P.; Pollack, A. D., and Baehr, G.: Pathology of Disseminated Lupus Erythematosus, Arch. Path. 32:569-631, 1941.

3. Fox, R. A.: Disseminated Lupus Erythematosus—an Allergic Disease? Arch. Path. 36:311-315, 1943.

4. Teilum, G.: Miliary Epitheloid-Cell Granulomas in Lupus Erythematosus Disseminatus, Acta path. et microbiol. scandinav. 22:73-79, 1945.

5. Teilum, G.: Pathogenetic Studies on Lupus Erythematosus Disseminatus and Related Diseases, Acta med. scandinav. 123:126-142, 1946.

6. Rich, A. R.: Hypersensitivity in Disease with Especial Reference to Periarteritis Nodosa, Rheumatic Fever, Disseminated Lupus Erythematosus and Rheumatoid Arthritis, Harvey Lect. (1946-1947) 42:106-147, 1947.

7. Bergstrand, H.: Morphological Equivalents in Polyarthritis Rheumatica, Periarteritis Nodosa, Transient Eosinophilic Infiltration of the Lung and Other Allergic Syndromes, J. Path. & Bact. 58:399-409, 1946.

8. Teilum, G.: Hyperglobulinemia, Periarterial Fibrosis of the Spleen, and the Wire Loop Lesion in Disseminated Lupus Erythematosus in Relation to Allergic Pathogenesis, Am. J. Path. 24:409-427, 1948.

9. Smith, J. F.: The Kidney in Lupus Erythematosus, J. Path. & Bact. 70:41-51, 1955.

10. Hargraves, M. M.; Richmond, H., and Morton, R.: Presentation of 2 Bone Marrow Elements: The "Tart" Cell and the "L. E." Cell, Proc. Staff Meet. Mayo Clin. 23:25-28, 1948.

11. Klemperer, P.; Gueft, B.; Lee, S. L.; Leuchtenberger, C., and Pollister, A. W.: Cytochemical Changes of Acute Lupus Erythematosus, Arch. Path. 49:503-516, 1950.

12. Gross, L.: The Heart in Atypical Verrucous Endocarditis (Libman-Sacks), in Contributions to the Medical Sciences in Honour of Dr. Emanuel Libman by His Pupils, Friends and Colleagues, New York, The International Press, 1932, Vol. 2, p. 527.

13. Gueft, B., and Laufer, A.: Further Cytochemical Studies in Systemic Lupus Erythematosus, A. M. A. Arch. Path. 57:201-226, 1954.

14. Teilum, G.; Engbaek, H. C.; Harboe, N., and Simonsen, M.: Effects of Cortisone on Experimental Glomerulonephritis, J. Clin. Path. 4:301-315, 1951.

15. Teilum, G.: Periodic Acid-Schiff-Positive Reticulo-Endothelial Cells Producing Glycoprotein: Functional Significance During Formation of Amyloid, Am. J. Path. 32:945-959, 1956.

16. Muehrcke, R. C.; Kark, R. M.; Pirani, C. L.; Pollak, V. E., and Steck, I. E.: Histological and Clinical Evolution of Lupus Nephritis, Ann. Rheumat. Dis. 14:371-377, 1955.

17. Kaiser, I. H.: The Specificity of Periarterial Fibrosis of the Spleen in Disseminated Lupus Erythematosus, Bull. Johns Hopkins Hosp. 71:31-43, 1942.

18. Bichel, J.; Bing, J., and Harboe, N.: Another Case of Hyperglobulinemia and Affection of the Central Nervous System, Acta med. scandinav. 138:1-14, 1950.

19. Eversole, S. L.: Cases of Disseminated Lupus Erythematosus Diagnosed as Idiopathic Thrombocytopaenic Purpura, Bull. Johns Hopkins Hosp. 96:210-222, 1955.

20. Robertson, T.: Plasmacytosis and Hyperglobulinemia as Manifestations of Hypersensitivity: A Postmortem Study of 2 Cases with Hypersensitivity Probably Due to Sulfadiazine, Am. J. Med. 9:315-329, 1950.

21. Klein, H., and Block, M.: Bone Marrow Plasmocytosis: A Review of 60 Cases, Blood 8: 1034-1041, 1953.

22. Teilum, G.: Cortisone-Ascorbic Acid Interaction and the Pathogenesis of Amyloidosis: Mechanism of Action of Cortisone on Mesenchymal Tissue, Ann. Rheumat. Dis. 11:119-135, 1952.

23. Teilum, G.: Studies on Pathogenesis of Amyloidosis: II. Effect of Nitrogen Mustard in Inducing Amyloidosis, J. Lab. & Clin. Med. 43: 367-374, 1954.

24. Rinehart, J. F.; Farquhar, M. G.; Jung, H. C., and Abul-Haj, S. K.: The Normal Glomerulus and Its Basic Reactions in Disease, Am. J. Path. 29:21-31, 1953.

25. Altschuler, C. H., and Angevine, D. M.: Histochemical Studies on the Pathogenesis of Fibrinoid Am. J. Path. 25:1061-1077, 1949.

26. Brunson, J. G., and Davis, R. L.: Systemic Fibrinoid Diseases: Similarity to Experimental Lesions in Rabbits, A. M. A. Arch. Path. 60:593-609, 1955.

27. Worken, B., and Pearson, R. D.: Hematoxylin Bodies Associated with Allergic Angiitis in Absence of Lupus Erythematosus, A. M. A. Arch. Path. 56:293-300, 1953.

28. Meacham, G. C., and Weisberger, A. S.: Unusual Manifestations of Disseminated Lupus Erythematosus, Ann. Int. Med. 43:143-152, 1955.

Influence of Muscle Section on Exostoses of Lathyric Rats

CHRISTOPHER J. HAMRE, Ph.D., and VERNON L. YEAGER, Ph.D., Grand Forks, N. D.

It has long been known that exostoses form a prominent skeletal deformity of rats fed a diet containing meal of the sweet pea Lathyrus odoratus.[1] Though a number of investigators have noted that the exostoses of lathyric rats appear to form at points of attachment of muscles to the skeleton,[1-10] only recently have investigators attempted to identify muscles which appear to be related to regularly occurring and precisely located exostoses.[6] Because of the possible relation of exostoses and skeletal muscles of lathyric rats, it has been suggested that exostosis formation may be due to the pull of spastic muscles on periosteum and bone, the lifting and detachment of the periosteum from the bone by muscle pull, or other changes of the periosteum and bone.[2,3,6,7]

We have demonstrated that an exostosis located at the common insertion to the femur of the pectineus and adductor longus muscles appears the earliest and develops the most rapidly of all of the exostoses of adult rats fed a sweet-pea diet. This exostosis, identified as the pectineus-adductor longus exostosis, was found to take origin during the first day of Lathyrus feeding by a process of proliferation and reorganization of the tissues of the inner layer of the portion of the periosteum which provide attachment for those muscles. Further, the new tissues of that portion of the periosteum, beginning on the seventh or eighth day of sweet-pea feeding, were found to give origin by processes of intramembranous osteogenesis to both the osseous and the marrow elements of the exostosis.[11]

Since the pectineus-adductor longus exostosis has been found to be formed directly from the tissues of the periosteum which serve for muscle attachment, the suggestion that skeletal muscles influence the formation of exostoses by lathyric rats appears to be a logical one.[11] It is possible that to produce exostoses the Lathyrus factor of sweet peas must be accompanied by a second or muscle factor. Tension or pull exerted on tissues which provide attachment for muscles may be the second factor. This is the report of a study designed to determine if elimination of the possible muscle factor by transection of muscles influences the formation of exostoses by lathyric rats.

Materials and Methods

To eliminate developmental and growth changes of the skeleton, only adult animals were used for this experiment.* Eleven female rats with an average body weight of 269 gm. and a range in body weight of 256 to 321 gm. were subjected to the experimental procedures described below.

The muscles of the animals to be sectioned were selected on the basis of being associated with exostoses which present a fairly constant shape and are fairly clearly separated from neighboring exostoses. The exostosis on the shaft of the femur at the common insertion of the pectineus and adductor longus muscles presents those characteristics. Also, an exostosis, or sometimes two exostoses, on the femur at the insertions of the iliacus and psoas major muscles exhibits those characteristics. The pectineus, adductor longus, iliacus, and psoas major muscles were, therefore, selected for transection. It was assumed that if muscle pull and tension are required for formation of exostoses by lathyric animals, transection of those

Submitted for publication May 3, 1957.

Department of Anatomy, University of North Dakota School of Medicine.

This investigation was supported by research grant C-2153(C4) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

* Obtained from Holtzman Rat Company, Madison, Wis.

muscles would result in modification and perhaps elimination of the two exostoses related to them.

Brief descriptions of the pectineus, adductor longus, iliacus and psoas major are presented here for three reasons: first, to permit the point of transection of each muscle to be clearly indicated; second, because the descriptions of the muscles will be of assistance in describing the results of the transections of the muscles to be given below, and third, because we cannot accept the identification and descriptions of those muscles, particularly the iliacus and psoas major, presented in the chief reference work on the anatomy of the white rat.[12] We have found the descriptions of the wood rat in almost all respects to apply to the muscles of the white rat.[18]

The pectineus muscle of the rat, as described in an earlier paper by us, takes origin on the pubis from the pectineal process and a narrow area ventral to the acetabulum. The body of the muscle extends laterally and inferiorly toward the femur and is composed of short lateral and long medial fibers. The short lateral fibers, beginning at the level of the lesser trochanter and continuing distally, are inserted on the medial surface of the femur. The medial long fibers of the pectineus muscle extend to and are inserted on the femur near the junction of the middle and distal third of the shaft.

The adductor longus muscle is placed immediately posterior to and borders on the pectineus muscle. It takes origin on a long narrow strip bordering on the symphysis pubis. It is a thin band-like muscle and distally narrows to form a thin flat tendon for insertion on the femur adjacent to the insertion of the medial portion of the pectineus muscle. Only the insertion of the medial long fibers of the pectineus muscle and the insertion of the adductor longus muscle are associated with the pectineus-adductor longus exostosis.

The iliacus muscle of all white rats dissected by us was found to take origin from the ventral portion of the crest and entire ventral margin of the ilium. It is a broad stout muscle, whose proximal portion lies ventral to the ilium but dorsal to the psoas major muscle. The distal portion of the muscle lies lateral to the pectineus muscle and between the pectineus muscle and the proximal end of the femur. Though the fibers of the iliacus muscle which take origin on the posterior portion of the margin of the ilium are attached directly to the femur, the greater portion of the muscle continues into a short broad musculotendinous bundle for insertion on the root of the lesser trochanter immediately anterior to the insertion of the quadratus femoris muscle.

The white rats of this experiment, and all other white rats examined by us, have not possessed a slender element of the psoas muscle complex placed in the position of the psoas minor described and illustrated for the wood rat.[18] Neither have we found elements of the psoas muscle which are inserted on the iliopectineal eminence (psoas process) or other portion of the innominate bone. We have concluded, therefore, that none of our animals have possessed a psoas minor muscle and that the psoas muscle complex of the white rat must be identified as being the psoas major muscle.

The psoas major muscle is of large size and is composed of two divisions separated from each other by thin fascial tissues and the femoral nerve. Of these the medial division forms the greater part of the muscle and takes origin from the lateral surface of bodies of the 13th thoracic to the 6th lumbar vertebrae, from the intervening fibrocartilages, and from poorly formed tendinous arches over the lumbar veins and arteries. It frequently has origin on the body of the first sacral vertebra. This division of the muscle also has origin by thin narrow slips from the tips of the transverse processes of the second, third, and fourth lumbar vertebrae. The various elements of the medial division of the psoas major muscle converge to form a broad stout body which continues posteriorly into a thick musculotendinous bundle inserted on the medial surface of the femur at the root of the lesser trochanter proximal and adjacent to the insertion of the iliacus muscle. The body of the second division of the psoas major muscle is placed lateral to the medial division but at its origin is dorsal or deep to the medial division. It takes origin by four or more slips from the tip of the transverse process of the fourth lumbar vertebra, from the bodies of the fourth and fifth lumbar vertebrae and their intervening intervertebral disk, and from the ventral surface of the quadratus lumborum muscle. This division of the psoas major muscle possesses a thin flat tendon and though it may reach and become attached to the femur with the iliacus muscle, it commonly is inserted on the lateral surface of the iliacus muscle. Proximal to their insertion, the two divisions of the psoas major muscle are, therefore, separated from each other by the terminal end of the iliacus muscle. Only the insertions of the psoas major and iliacus muscles are related to the iliacus-psoas major exostoses.

To permit each animal to serve as its own control, only the pectineus, adductor longus, iliacus, and psoas major muscles of the left side were transected. After the animals were given pentobarbital (Nembutal) anesthesia, an incision extending from the knee to the inguinal region was made through the skin of the left thigh, the skin was reflected, and the muscles were located and defined. To avoid injury to the exostosis-producing tissues of the periosteum at their insertion, the medial portion of the pectineus and the adductor

longus muscle were transected near the beginning of the tendon of insertion of the latter muscle. The iliacus and psoas major muscles were exposed from the lateral surface without disturbing the inguinal ligament or entering the peritoneal cavity, and, in all cases without damaging the femoral nerve, the two muscles were transected at the beginning of their musculotendinous insertion. The manner and position of transection of the various muscles left the tendon of insertion, or stumps of musculotendinous insertions, attached to the femur at the sites of origin of the pectineus-adductor longus exostosis and the iliacus-psoas major exostoses.

After transection of the muscles, 10 of the rats were fed a diet composed of equal parts of meal of Purina Laboratory Chow and meal of the sweet pea Lathyrus odoratus. The remaining animal was fed Purina Laboratory Chow only. All animals were separately caged and each day were weighed and given fresh food and water. At the end of each week the animals were examined for the presence of exostoses and other symptoms of lathyrism. Of the animals fed the lathyrogenic diet, three were killed after 6 weeks; two, after 12 weeks; one, after 13 weeks, and four, after 14 weeks of the diet. The control animal was killed after 21 weeks. All animals were killed with ether, skinned, and subjected to autopsy examination, and the entire carcass, with appendages extended, was fixed and preserved in a 10% formalin solution. The carcass of each animal was examined for the presence of exostoses or other abnormalities of the skeleton. The condition of the muscles of the thigh of the posterior appendages was noted, and the muscles, as far as required, were removed and the condition of the exostoses of the innominate bones and femora carefully examined.

Observations and Results

The control rat fed the Purina Laboratory Chow diet, after recovery from the operation of muscle section, was healthy and active throughout the period of the experiment. Also, after recovery from the operation the animal showed no lameness or altered movements of the left posterior appendage, thereby indicating that compensation for the loss of function of the sectioned muscles had occurred. At autopsy the transected left adductor longus, iliacus, psoas major, and medial portion of the pectineus muscles were found to have undergone marked atrophy. The skeleton of the animal was found to be free of exostoses or other abnormalities.

All rats fed the lathyrogenic diet recovered rapidly from the operation of myotomy and for some weeks after the operation, like the control animal, showed normal movements and compensation for loss of function of the transected muscles of the left posterior appendage. All rats fed the lathyrogenic diet later developed the common symptoms of lathyrism, i. e., inactivity, lameness, awkward gait, and limitation of some movements of all appendages. By the end of the experiment all animals fed the sweet-pea diet showed some degree of permanent abduction of the posterior appendages, which in all cases was greater for the intact right appendage than for the left appendage whose muscles had been transected. For all animals fed the diet for 12, 13, and 14 weeks, the knee was fairly rigid and the leg partially flexed on the thigh. The thorax was normal in shape for all animals except one fed the diet for 12 weeks and one fed the diet for 13 weeks, whose thorax was markedly dorsoventrally flattened. None of the animals fed the lathyrogenic diet for six weeks showed curvature of the back or other signs of abnormality of the spinal column. However, all animals fed the diet for 12 or more weeks exhibited dorsal curvature of the lumbosacral region of the back. Four of the animals fed the diet for 12 weeks or longer showed some degree of ventral curvature of the cervicothoracic region of the back.

Because the adductor longus, iliacus, and psoas major muscles possess well-defined tendinous or musculotendinous insertions, transection of those muscles was readily carried out. Autopsy examination and examination of the preserved carcasses showed transection of those muscles of all animals to have been successfully accomplished. Atrophy of those muscles, except as noted below, was marked or complete. For one animal the proximal end of the adductor longus muscle, shown in Figure 1, was fairly large, triangular in shape, and incompletely atrophied. The proximal cut end of that muscle was found to have acquired

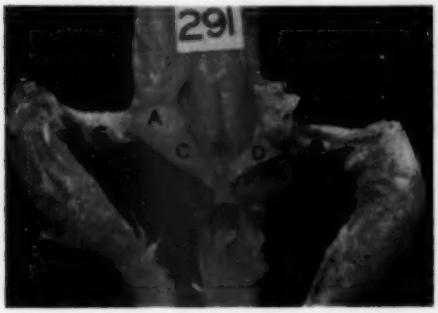


Fig. 1.—Ventral view of posterior appendages of a rat fed sweet-pea diet for six weeks. *A*, pectineus muscle, medial division. *B*, pectineus muscle, lateral division. *C*, adductor longus muscle, intact. *D*, adductor longus, sectioned. *E*, quadratus femoris. *F*, obturator externus. *G*, pectineus-adductor longus exostosis.

strong attachment to the surface of the adductor magnus muscle. The remnant of the adductor longus muscle was not associated with an exostosis.

Autopsy examination and dissection of the preserved specimens showed transection, with resultant atrophy, of the medial division of the pectineus muscle to have been successfully accomplished. However, because the medial and lateral divisions of the muscle are not sharply separated from each other, transection of the medial division was not as precise as for the other muscles. No attempt was made to transect the lateral portion of the pectineus, and, as shown in Figure 1, that portion of the muscle remained intact, functional, and a potential exostosis-producing muscle.

Examination of the preserved specimens showed all animals fed the sweet-pea diet to possess a large number of exostoses, thereby demonstrating that the diet employed was an effective lathyrogenic diet. Careful examination also showed the various exostoses to be placed at points of attachment of muscles to the skeleton. Excluding poorly defined exostoses and those related to the transected muscles, well-developed exostoses related to the origin or insertion of muscles were found at the following points on the skeleton: angles of the mandible, mandible at the insertion of the digastric muscles, lambdoidal ridge and neighboring portions of the skull, spine of the scapula, deltoid ridge of the humerus, extensor tubercle of the humerus, point of insertion of the pronator teres to the radius, greater and lesser trochanters of the femur, gluteal or third trochanter of the femur, crest of the tibia, and the posterior margin of the ribs at the insertions of the longissimus dorsi muscle. An exostosis was present on the head of the ulna of one animal fed the lathyrogenic diet for 14 weeks. One animal possessed an abnormal, dorsally curved sternum. The two animals with dorsoventrally flattened thorax exhibited enlargement of some costochondral junctions. Though cervicothoracic and lumbosacral curvatures were present in the living animals, grossly visible changes could not be identified for the vertebral column of preserved specimens.

Figure 1, a photograph of the innominate bones and femora of an animal fed the lathyrogenic diet for 14 weeks, can serve as a representative illustration of the condition of the exostoses related to the posterior appendages of all animals fed that diet. The exostosis located on the medial surface of the right femur at the root of the deformed lesser trochanter received the insertion of the intact right iliacus and psoas major muscles. This exostosis is shown to be composed of two parts separated by a groove but joined to each other anteriorly. The proximal osseous mass was formed at the point of insertion of the medial division of the psoas major muscle, while the distal mass was formed at the combined insertion of the iliacus muscle and lateral division of the psoas major muscle. Also shown in the Figure, exostoses were not present on the left femur at the insertion of the transected left iliacus and psoas major muscles. Since the left femur of all animals fed the lathyrogenic diet lacked iliacus-psoas major exostoses, transection of the left iliacus and psoas major muscles effectively prevented formation of those exostoses.

All animals fed the lathyrogenic diet possessed a well-developed pectineus-adductor

longus exostosis on the right femur at the insertion of the intact adductor longus muscle and intact medial portion of the pectineus muscle. For all animals fed the diet, as shown in Figure 1, the exostosis is a large crater-like osseous mass whose cavity is located at its proximal surface. In contrast, for all Lathyrus-fed animals no comparable exostosis was found on the left femur at the insertion of the transected adductor longus muscle and transected medial portion of the pectineus muscle. Two of the Lathyrus-fed animals possessed a low thin ridge of bone on the middle of the shaft of the left femur, but dissection showed the ridge of bone to be related to the insertion of the longest fibers of the lateral portion of the pectineus muscle. The ridge-shaped exostosis of the left femur of the two animals was, therefore, not comparable to the pectineus-adductor longus exostosis. Transection of the medial portion of the pectineus muscle and transection of the adductor longus muscle for all experimental animals prevented formation of a pectineus-adductor longus exostosis, and because of the absence of exostoses, the distal portion of the shaft of the left femur was slender, cylindrical, and normal in shape.

Though transection of the left adductor longus muscle and the medial division of the left pectineus muscle prevented formation of a pectineus-adductor longus exostosis on the left femur, the left femur also in other features did not respond typically to Lathyrus stimulation. The proximal portion of the shaft was broader than normal and also broader than the comparable region of the right femur. The narrow ridge of bone noted above as occurring on the left femur of two animals was located on the posterior margin of the broad region of the shaft. The medial surface of the broad region of the shaft for all animals was roughened by irregular exostoses. Examination of the left femur showed the above-described three structures to be located at the insertion of the intact lateral division of the pectineus muscle. Further, the left femur possessed an exostosis at the insertion of the adductor brevis on the posterior surface of the gluteal trochanter, where exostoses commonly do not occur. Also, as shown in Figure 1, the left femur possessed a prominent exostosis on the lesser trochanter at the insertion of the quadratus femoris muscle. Similar structures were not present or only poorly developed on the right femur, which received insertion of intact pectineus and adductor longus muscles.

The left innominate bone, when compared to the right innominate bone, like the femur, did not respond typically to Lathyrus stimulation. The ilium at the origin of the sectioned iliacus muscle was narrower than the right ilium, which gave origin to the intact iliacus muscle. The exostosis at the origin of the left adductor magnus muscle was larger than for the right adductor magnus muscle. The exostosis of the ischium at the origin of the left quadratus femoris muscle was larger for the left than for the right innominate bone. Also, for some animals the exostosis at the origin of the left pectineus muscle was larger than for the right pectineus muscle. Figure 1 is of a specimen showing a larger exostosis for the origin of the right than for the left pectineus muscle.

An observation of this study, though not bearing directly on the problem of the influence of transection of muscles on formation of exostoses, appears to be sufficiently important to be noted here. It was noted above that the posterior appendages of rats in advanced lathyrism are permanently in the position of marked abduction. This may suggest that powers of adduction had been lost and that paralysis of the appendages had appeared. However, as is clearly shown in Figure 3, the presence of large fibroma-like masses of proliferating connective tissue related to the iliacus-psoas major exostosis and the exostosis at the origin of the pectineus muscle make adduction of the posterior appendages mechanically impossible. When the tumor-like masses reach large size, they border directly on each other

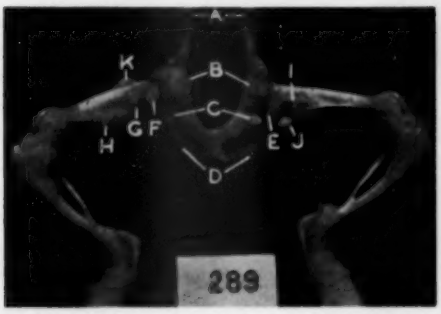


Fig. 2.—Ventral view of posterior appendages of a rat fed sweet-pea diet for 14 weeks. *A*, origin of iliacus muscle. *B*, origin of pectineus muscle. *C*, origin of adductor magnus muscle. *D*, origin of quadratus femoris muscle. *E*, insertion of quadratus femoris muscle. *F*, insertion of psoas major muscle. *G*, insertion of iliacus muscle. *H*, pectineus-adductor longus exostosis. *I*, insertion of lateral division of pectineus muscle. *J*, exostosis in insertion of adductor brevis to gluteal trochanter. *K*, exostosis of gluteal trochanter, projecting forward.

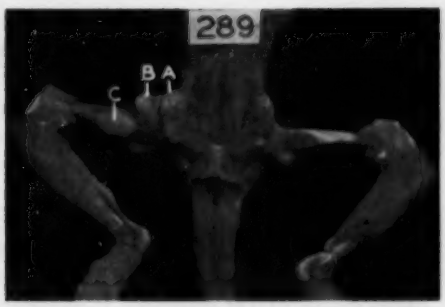


Fig. 3.—Ventral view of appendages of a rat fed sweet-pea diet for 14 weeks. *A*, tumor-like mass at origin of pectineus muscle. *B*, tumor-like mass at insertion of iliacus and psoas major muscles. *C*, pectineus-adductor longus exostosis.

and, as shown in Figure 3, acquire flattened surfaces for anteroposterior movement of one on the other. Though composed, as described in our earlier paper,[11] of soft proliferating fibroma-like tissue, the masses are not compressible, and the posterior appendages are forced outward into a position of permanent abduction. It was also noted above that in advanced lathyrism abduction for the right posterior appendage was greater than for the left posterior appendage, whose muscles had been transected. Transection of the iliacus and psoas major muscles has, as shown in Figure 3, eliminated the iliacus-psoas major fibromatoid mass, and though the mass at the origin of the left pectineus muscle is present, it alone can produce but moderate abduction of the left posterior appendage. It is clear that the tumor-like masses of connective tissue exercise a greater influence on movements of the appendages than do the comparatively much smaller osseous bodies they produce.

Comments

Early investigators of lathyrism in the rat, by recording observations that exostoses appeared to form at points of attachment of muscles to the skeleton, implied that muscles in some manner may influence formation of exostoses in that disease. Our observations that exostoses are formed by the periosteum at the point of junction of muscle and bone offered strong evidence that some relation of muscles to exostosis formation does exist.[11] Since muscles by their contraction must exert mechanical tension or pull on the skeleton and periosteum, it appeared that muscle tension may be a factor in exostosis production. The present experiment has demonstrated that transection of muscles, and thereby elimination of tension on exostosis-producing tissues, does prevent origin and development of exostoses directly related to the transected muscles. Absence of exostoses after muscle section, even though the periosteal exostosis-producing tissues are intact and uninjured, provides evidence that stimulation of those tissues by the sweet-pea factor alone cannot produce exostoses. It may be concluded that simultaneous stimulation of periosteal exostosis-producing tissues by the sweet-pea factor and the muscle factor is required for active production of exostoses to occur. Muscles, therefore, appear to exercise a determinative influence on the origin and development of exostoses of lathyrism.

The occurrence of atypical exostoses and modification of structure and appearance of commonly occurring exostoses on the femur and innominate bone following section of muscles related to them provide additional evidence of the influence exerted by muscles

on exostosis production in lathyrism. The influence exercised in these instances appears to be related to the phenomenon of compensation for function lost by section of muscles. That compensation for lost function did occur was shown by the presence of normal movements of the left posterior appendage after our animals recovered from the operation of myotomy. The appearance of new exostoses and change of commonly occurring exostoses at the origin or insertion of the lateral division of the pectineus muscle, adductor magnus and adductor brevis muscles, and the quadratus femoris muscle suggest that those muscles assume the function of adduction lost by section of the adductor longus and medial division of the pectineus muscles. We believe that this phenomenon emphasizes the importance of muscle activity as a necessary factor for the production of exostoses by lathyric rats.

We have observed that exostoses do not appear at the same time for all muscles and that not all muscles, even in advanced lathyrism, become associated with exostoses. The fact that section of a limited number of muscles is accompanied by formation of new exostoses or change of commonly occurring exostoses related to the remaining intact muscles may offer an explanation for the delay in appearance or absence of exostoses at the attachments of some muscles. Section and inactivation of muscles must transfer work to remaining intact muscles of similar function, whose normal activity is not great enough to stimulate formation of exostoses. We suggest that the activity and work accomplished by some muscles is below that required for response to the sweet-pea factor and, therefore, remain without exostoses. We have undertaken experiments in which animals are subjected to enforced exercise as a test of the hypothesis that increased work and activity of muscles may induce widespread and stronger response to stimulation by the sweet-pea factor.

It may be pointed out here that many of the muscles related to the skeleton of the thigh of rats are deeply placed and, therefore, not readily transected. Also, some of the strongest exostosis-producing muscles have broad attachments to the femur, thereby making complete and precise transection difficult. However, inactivation of muscles by section of nerves which supply them also eliminates muscle tension stimulation on exostosis-producing tissues. Because of the possibility of obtaining more precise results, we have carried out a muscle denervation experiment as a companion to our present muscle section experiment. The results of that experiment, which are now being analyzed and prepared for publication, also point to muscles as important factors in the production of exostoses by lathyric rats.

Summary

To test the hypothesis that origin and development of exostoses by lathyric rats requires stimulation of exostosis-producing tissues by both the sweet-pea factor and muscle factor, possibly muscle tension, 11 adult rats were subjected to transection of muscles related to the pectineus-adductor longus and iliacus-psoas major exostoses of the femur. Only the iliacus, psoas major, and adductor longus muscles and the medial portion of the pectineus muscle of the left posterior appendage were sectioned. Ten of the animals subjected to muscle section were given a diet of equal parts of meal of the sweet pea Lathyrus odoratus and Purina Laboratory Chow. The animals were killed after 6, 12, 13, or 14 weeks on the lathyrogenic diet. The remaining animal was fed Purina Laboratory Chow and killed after 21 weeks.

Though the control rat remained free of exostoses, exostoses and other abnormalities of the skeleton appeared in all animals fed the lathyrogenic diet. Exostoses for those animals were typical in size and shape for all parts of the skeleton, excepting the left innominate bone and femur. Exostoses failed to appear at the insertion of tran-

sected muscles. Commonly occurring exostoses were increased in size, and new exostoses appeared at the attachments of some of the remaining muscles attached to the left innominate bone and femur. The results of this experiment demonstrate that the sweet-pea factor alone does not produce exostoses but that it can do so in the presence of tension of intact functional muscles.

Department of Anatomy, University of North Dakota School of Medicine.

REFERENCES

1. Geiger, B. J.; Steenbock, H., and Parsons, H. T.: Lathyrism in the Rat, J. Nutrition 6:427-442, 1933.

2. Robinson, J. J., and Bast, T. H.: Bone Changes Due to Lathyrism in Rats, Anat. Rec. 59:283-295, 1934.

3. Robinson, J. J.: Pathology Produced by Lathyrism in Rats, Master's Dissertation, University of Wisconsin, 1934.

4. Lewis, H. B.; Fajans, R. S.; Esterer, M. B.; Shen, C. W., and Oliphant, M.: The Nutritive Value of Some Legumes: Lathyrism in the Rat; the Sweet Pea (Lathyrus Odoratus), Lathyrus Sativus, Lathyrus Cicera and Some Other Species of Lathyrus, J. Nutrition 36:537-559, 1948.

5. Lee, J. G.: Experimental Lathyrism Produced by Feeding Singletary Pea (Lathyrus Pusillus) Seed, J. Nutrition 40:587-594, 1950.

6. Ponseti, I. V.: Lesions of the Skeleton and of Other Mesodermal Tissues in Rats Fed Sweet-Pea (Lathyrus Odoratus) Seeds, J. Bone & Joint Surg. 36-A:1031-1058, 1954.

7. Gerschon-Cohen, J., and McClendon, J. F.: Roentgenography of Osteoporosis Due to Lathyrism in the Rat: Effect of Rock Phosphate in the Diet, Radiology 64:727-730, 1955.

8. Hamre, C. J., and Yeager, V. L.: Influence of Tenotomy on Formation of Exostoses by Rats Fed Lathyrus Odoratus, Abstract, Anat. Rec. 124: 406, 1956.

9. Selye, H., and Bois, P.: Effect of STH on Experimental Lathyrism, Proc. Soc. Exper. Biol. & Med. 94:133-137, 1957.

10. Menzies, D. W., and Mills, K. W.: The Aortic and Skeletal Lesions of Lathyrism in Rats on a Diet of Sweet Pea, J. Path. & Bact. 73:223-237, 1957.

11. Yeager, V. L., and Hamre, C. J.: Histology of Lathyrus-Induced Exostoses of Rats: The Initial Changes at Tendon-Bone Junction, A. M. A. Arch. Path. 64:171, 1957.

12. Greene, E. C.: Anatomy of the Rat, Reprint of the Transactions of the American Philosophical Society, New Series, New York, Hafner Publishing Co., 1955, Vol. 27.

13. Howell, A. B.: Anatomy of the Wood Rat, Baltimore, Williams & Wilkins Company, 1926.

Elastic Fiber Alterations in Rats Treated with Lathyrus Odoratus

Histopathologic Study of Elastic Cartilage and of Elastic Fibers in Arteries and Membranes, with Special Reference to the Occurrence of Extra-Aortic Dissecting Aneurysms

DONALD G. WALKER, Ph.D., Baltimore

Histopathological studies of the various widely distributed lesions of lathyrism have disclosed alterations of the ground substance,[1-3,5] elastolysis,[2,4,6,7] and foci of fibroblastic proliferation.[5,7] Not only does the severity of the disease process vary inversely with the degree of maturity of the animal, but also the type and location of the lesion vary with age, and the younger the animal the more extensive and pleomorphic are the lesions. Although Ponseti's[5] original observation, that dissecting aneurysms cannot be produced in rats over 7 weeks of age, has been confirmed, there are differences of opinion regarding the extent to which the arterial system is subject to histopathologic alterations when the exposure to the Lathyrus factor is initiated at the time of weaning (usually three weeks after birth in rats). It has been reported repeatedly that the formation of aneurysms is limited strictly to the thoracic aorta.[1,4-6,8] Nonetheless, Walker and Wirtschafter[7] observed dissecting aneurysms in the abdominal as well as the thoracic portion of the aorta and elastic laminar disintegration even in the pulmonary and coronary arteries. In near-term rat embryos the mothers of which had been fed the sweet-pea diet during the last week of gestation,[9-11] fragmentation of the elastic laminae of the entire aorta is virtually complete.

Submitted for publication May 3, 1957.

Department of Anatomy, The Johns Hopkins University School of Medicine.

Although the histopathologic alterations that characterize the aortic lesions of lathyrism have been described, their significance in the dynamics of the disease process requires further elucidation. The histochemical studies reported by Churchill et al.[2] indicate that chondroitin sulfate B is present in abnormally high quantities in the thoracic aortae of rats that have been fed a Lathyrus odoratus diet from the time of weaning. The autoradiographic study with S^{35}, of Ponseti et al.,[12] did not bear out the findings of Churchill et al., since no greater content of S^{35} was observed in the aorta bearing the dissecting aneurysm than in that of the control. Since no constant relationship was apparent between the extent of elastic fiber disintegration and the quantity of associated interlaminar material, Churchill et al.[2] doubt that the latter is merely a product derived from the breakdown of elastic fibers.

The experimental destruction of the elastic fibers in the blood vessels of rabbits by injection of either ammonium hydroxide[13] or thyroxin[14] has been reported by Baló. Regeneration of elastic fibers was effected apparently by cells resembling fibroblasts. Throughout the aorta of rats fed the Lathyrus factor from the time of weaning clusters of cells similar to those in the lesions described by Baló were seen wedged in between the disintegrating laminae. With the evidence at hand it may not be assumed that these cells subserve either an elastolytic or an elastogenic function. However,

as a working hypothesis these fibroblasts may be regarded as elastogenic cells which are not able to complete the synthesis of elastin in the presence of Lathyrus toxin (β-aminoproprionitrile). The fact that elastic fiber alterations can be produced in the aorta only if the rats are less than 7 weeks old at the time treatment is instituted lends support to this hypothesis. The following experiment was undertaken in order to provide further elucidation as to the nature of the abnormally increased interlaminar substance and to ascertain whether or not more widespread elastic fiber deficiencies would be demonstrable if the Lathyrus factor were administered prior to weaning.

Methods and Materials

Diet.—A crude extract of finely comminuted L. odoratus seeds was prepared by simply combining the pea powder with two parts of water, shaking the suspension for 15 to 20 minutes, and filtering through a 12-ply gauze sponge. The filtrate was administered via stomach tube by means of a syringe and needle to which a short length (2 to 5 cm.) of polyethylene tubing was fitted as recommended by the manufacturer (Clay-Adams Co., Inc.). The size of needle and tubing was increased with the age of the animal, as was the volume of extract administered. For stomach-tubing rats up to 4 days of age a 30-gauge needle with PE-10 tubing was used. This tubing of very small bore necessitated refiltration of the extract through filter paper. For stomach-tubing rats 4 to 18 days of age a 22-gauge needle fitted with PE-50 tubing was used. About 0.25 ml. of extract per 10 gm. of body weight was administered three times a day (morning, noon, evening). Control rats received an equivalent volume of H_2O.

From time of weaning, at 17 days of age, until autopsy, 2 to 4 weeks later, all experimental rats were maintained on the pea diet. The normal controls, which from the time of weaning were housed separately from the experimental rats, received the following stock dietary mixture: whole wheat, 65.50%; casein, 15.00%; whole-milk powder, 10.00%; iodized NaCl, 0.75; $CaCO_3$, 1.50, and vitamin concentrate (Quadrex, of Nopco Chemical Co.) in an amount to give 1.25 U. S. P. units of vitamin D and 10.0 units of vitamin A per gram of diet.

A thorough mixture in 1:1 proportions of the control ration and finely ground seeds of the pea, L. odoratus, constituted the experimental diet.

Animals.—Six newborn litters, each consisting of three male and three female albino rats (obtained from Carworth Farms, Inc.), were used in this experiment. In each litter two rats (one of each sex) were reared as controls; the other four littermates received the pea extract.

Autopsy.—One litter was killed at 4 weeks of age; two, at 5 weeks of age, and the remainder, at 6 weeks of age. With use of ether anesthesia the following membranes were excised and outspread upon slides: (*a*) subcutaneous areolar connective tissue of the scalp, anterior abdominal wall, and left thigh; (*b*) mesoappendix; (*c*) mesentery proper; (*d*) falciform ligament, and (*e*) capsule of the left kidney. Upon death of the animal additional spreads were made of the pericardium, dura mater, and periosteum of the calvaria. A list of the major arteries that were examined at autopsy and excised for histologic study will be found in the presentation of results. All the membranes and vessels were fixed in Zenker's solution and formol.

Histologic Preparation.—The spreads of membranes were washed and stained for elastic fibers according to the method of Verhoeff, and counterstained with Van Gieson's picrofuchsin. The vessels were embedded in tissuemat, oriented, and cut in order to obtain both longitudinal and transverse sections 6μ in thickness. From every block at least eight serial sections were mounted separately so that each could be stained by one of eight different procedures, including (1) Verhoeff's hematoxylin and Van Gieson's picrofuchsin; (2) Taenzer-Unna's orcein and Harris' hematoxylin; (3) Heidenhain's azan (H. A.); (4) Mallory's phosphotungstic acid hematoxylin (P. A. H.); (5) 0.03% toluidine blue solution at pH 6.8 as used by Churchill et al.[9]; (6) Foot-Menard's diamine silver carbonate method for reticulum; (7) McManus' periodic acid-Schiff (P. A. S.) procedure with and (8) without pretreatment in 5% phenylhydrazine hydrochloride, as recommended by Lillie.[10]

Results

A. Autopsy Findings.—When the contents of the undissected abdomen of the L. odoratus-treated rats were examined grossly, the mesenteries and gastrointestinal tube appeared to be abnormally flaccid and of redundant length. The stomach and cecum were at least twice as voluminous in the experimental rats as in the controls. The colon was beset with numerous diverticula, which usually were distended with the unassimilated residue derived from the pea diet. When microscopic examination was conducted in vivo on selected portions of the mesentery proper and mesoappendix

(mesocecum), it became evident that in the experimental animals the elastic fiber content of these membranes was conspicuously diminished and the smaller radicles of the mesenteric circulation were abnormally fragile and unusually prone to petechia formation and thrombosis. The deficiency of elactic fibers was confirmed in fixed and stained preparations of these and a variety of other membranes removed from L. odoratus-treated rats. Without sufficient elastic fiber support the mesenteries apparently had become overstretched and the hollow viscera, overdistended.

One animal autopsied a few minutes after death was found to have gangrene of a large portion of the jejunum. This finding prompted a careful examination of the mesenteric vessels and led to the disclosure in the superior mesenteric artery of a dissecting aneurysm which involved the vessel from its origin for about 3 cm. along its length. In fact, it developed that the arterial tree of this particular animal was very extensively involved. Aneurysms not only dissected through the entire length of the aorta and into the superior mesenteric artery but also into both common carotid arteries, the innominate, the left subclavian, both renal, and both common iliac arteries. Of the other 18 experimental rats carefully examined at autopsy, 7 were found with aneurysms in major arteries other than the aorta. The number of animals with aneurysms in a given vessel is indicated in parentheses as follows: thoracic aorta (18), abdominal aorta (11), coronary arteries (2), innominate artery (5), right common carotid (1), left common cartoid (3), right subclavian (1), left subclavian (2), celiac axis (4), hepatic artery (1), superior mesenteric artery (3), both renal arteries (3), left iliolumbar artery (1), both common iliac arteries (7), both external iliac arteries (3), both internal iliac arteries (1). No isolated aneurysm was found in any of the peripheral vessels. The path of every aneurysm could be traced back to the aorta. Therefore, it would seem likely that all aneurysms had originated in the aorta and gained access to peripheral vessels secondarily by direct extension. Though the pulmonary arteries of experimental rats were free of actual aneurysms, the same histologic alterations seen in the other major arteries were consistently disclosed in the pulmonary arteries.

B. Histologic Findings.—Photomicrographs of a few of the different vessels in which aneurysms had developed are shown

Fig. 1.—Involving this intercostal artery for at least two-thirds of its circumference is a dissecting aneurysm that has split the external elastic lamina away from the tunica media. The abnormal, black-stained deposits seen close to the peripheral aspect of the internal elastic membrane are believed not to represent true elastin. Transverse section, Verhoeff and Van Gieson's stain; reduced ⅔ from mag. × 125.

Fig. 2.—A normal intercostal artery. Transverse section, Verhoeff and Van Gieson's stain; reduced ⅔ from mag. × 125.

Fig. 3.—The dissecting aneurysm seen in this innominate artery was found to have involved the vessel throughout its length. Transverse section, Verhoeff and Van Gieson's stain; reduced ⅔ from mag. × 125.

Fig. 4.—This comparatively enormous aneurysm had dissected a path just deep to the adventitia from the aorta along this superior mesenteric artery for a greater portion of its length and eventually so embarrassed the circulation to a large portion of the small intestines that the latter became gangrenous. Longitudinal section, Verhoeff and Van Gieson's stain; reduced ⅔ from mag. × 100.

Fig. 5.—The common iliac arteries shown here just proximal and just distal to the terminal bifurcation of the vessel again bear evidence that the Lathyrus-produced aneurysms may extend far beyond the thoracic aorta. The nodular lesion eccentrically placed in the media of the internal iliac artery at the extreme right side of the figure is very similar to that shown at higher magnification in Figure 9. Transverse section, Taenzer-Unna's orcein and Harris' hematoxylin; reduced ⅔ from mag. × 100.

Fig. 6.—The left subclavian artery of an experimental rat in which the path of destruction wrought by the aneurysm is limited to the outer portion of the tunica media. Longitudinal section, Verhoeff and Van Gieson's stain; reduced ⅔ from mag. × 100.

Fig. 7.—The left subclavian artery of a normal control rat. Longitudinal section, Verhoeff and Van Gieson's stain; reduced ⅔ from mag. × 100.


2
3
4

in Figures 1, 3, 4, 5, and 6. The blood-filled cleft that identifies the course along which the aneurysm had dissected was nearly always seen just deep to the external elastic membrane, though why the outer media should be particularly vulnerable to the disruptive forces of an aneurysm remains to be clarified. Normal vessels are shown in Figures 2 and 7.

Disintegration of elastic fibers and an infiltration by fibroblast-like cells were constantly found in the walls of aneurysmal clefts. Of greater interest is the fact that these same histologic alterations were observed occasionally in small and medium-sized arteries in which no aneurysm had formed. In Figure 8 the left iliolumbar artery of an experimental rat (W 99) is shown in cross section, stained with Verhoeff's hematoxylin and Van Gieson's picrofuchsin. The internal elastic membrane has been interrupted, and the free ends have a frayed appearance. Throughout the tunica media of the vessel there is a high concentration of small fibrillar elements and finely granular material, all of which is stained black and accordingly should represent elastin. However, since the specificity of Verhoeff's hematoxylin for elastin has been doubted, the impressions gained by the use of certain other connective tissue stains as suggested by Gilman et al.[16] should be considered. When a section cut from the same iliolumbar artery as that just described was stained with Heidenhain's azan, most of the fibrils and granules that appeared black with Verhoeff's hematoxylin were now seen to be stained blue and, accordingly, would not represent true elastin (Fig. 9). In fact, only a few short thin remnants of the internal elastic membrane still remained to give the staining properties of true elastin. The several closely packed nests of cells located along the inner margin of the tunica media are much more readily seen in the section stained with H. A. (Fig. 9) than in that stained by the method of Verhoeff and Van Gieson (Fig. 8). From the normal smooth-muscle cells of the tunica media the newly differentiated cells associated with the altered elastic fibers are easily distinguished by their more or less radial orientation and their colorless clear cytoplasm. The observations made on sections stained with either orcein or Mallory's P. A. H. tended to bear out the impressions noted in the H. A.-stained section. In orcein-stained sections the bulk of the fibrillar and granular material in the vessel wall was stained a light reddish-brown, and in the P. A. H.-stained sections the corresponding elements were stained pale orange, findings which indicate a severe degree of "elastotic degeneration."[16] A lesion similar to that just

Fig. 8.—In this left iliolumbar artery of an experimental rat discontinuities of the internal elastic lamina are evident. The smaller fragments of the lamina have a frayed appearance. Transverse section, Verhoeff and Van Gieson's stain; reduced ⅔ from mag. × 250.

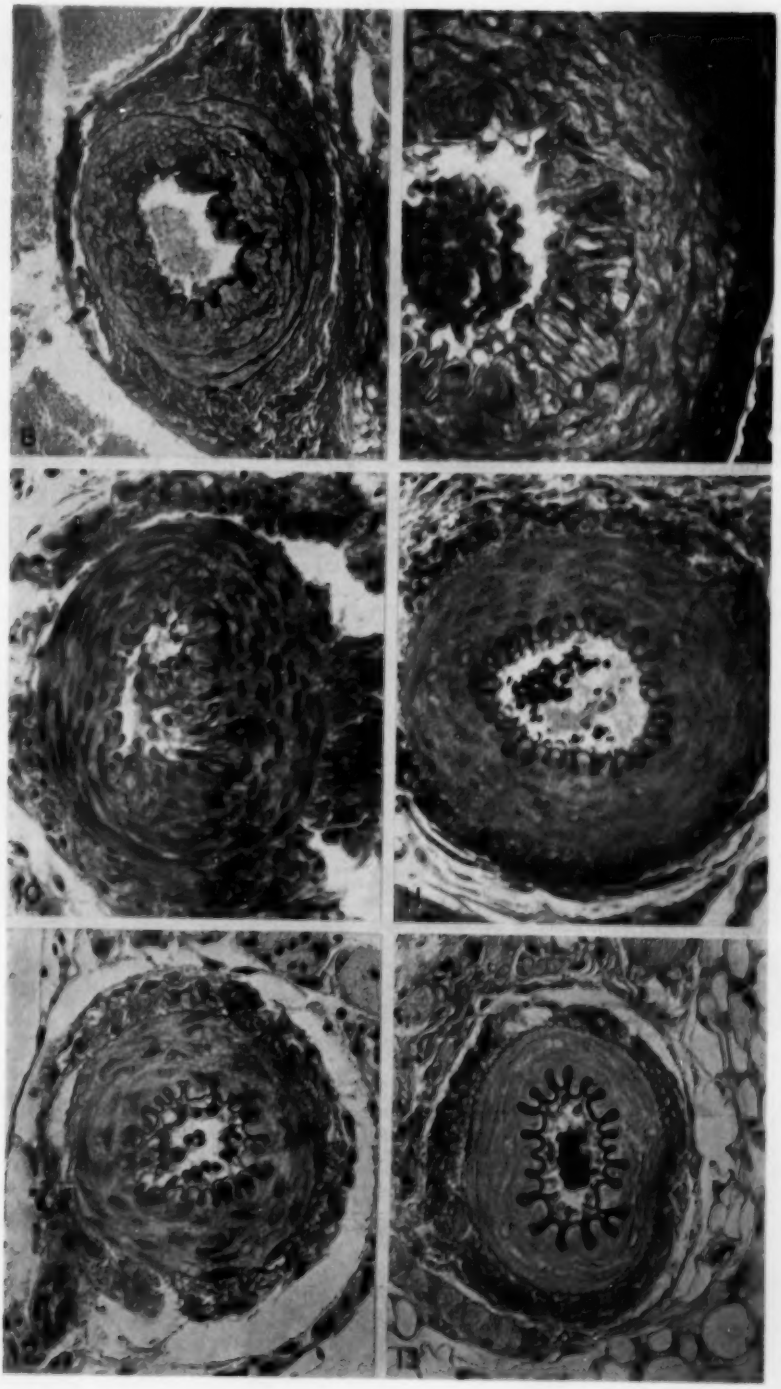
Fig. 9.—This is a section serially cut from the same block as that shown in Figure 8. Even less of the internal elastic lamina remains than is evident in the section stained by the Verhoeff method by which "pseudo" as well as "true" elastin are stained black. Nests of cells (presumably fibroblasts) are seen closely packed in the areas where the internal elastic lamina is most conspicuously deficient. Transverse section, Heidenhain's stain; reduced ⅔ from mag. × 500.

Fig. 10.—In this femoral artery of an experimental animal an exuberant proliferation of fibroblasts has produced a nodule that has filled in a large portion of the vessel's lumen. Nothing remains of the internal elastic lamina in the area of the intima occupied by the nodule. The multiple cytoplasmic processes and prominent nucleoli characterize the otherwise faintly stained cells composing the lesion. Transverse section, Mallory's phosphotungstic acid hematoxylin stain; reduced ⅔ from mag. × 400.

Fig. 11.—A normal femoral artery of the littermate control to the experimental rat a femoral artery of which is shown in Figure 10. Transverse section, phosphotungstic acid hematoxylin stain, reduced ⅔ from mag. × 400.

Fig. 12.—In experimental animals erosion, even to the extent of perforation, of the internal elastic lamina was often evident in small arteries, such as the mesenteric radicle illustrated here. However, such fine alterations were effectively masked by Verhoeff's method. Transverse section, Mallory's phosphotungstic acid hematoxylin stain; reduced ⅔ from mag. × 500.

Fig. 13.—A small mesenteric artery obtained from a normal control animal. Transverse section, Mallory's phosphotungstic acid hematoxylin stain; reduced ⅔ from mag. × 500.



described but representing a more advanced stage of the pathological process is seen in Figure 10, a photomicrograph of the femoral artery of another experimental animal (W 91). Shown in Figure 11 is a corresponding section of a normal femoral artery which, like that of the experimental rat, had been stained with Mallory's P. A. H. The lesion consists of a nodular mass of cells, some of which possess distinct processes and prominent nucleoli but which, otherwise, are of variable size, shape, and orientation and are only faintly stained. The lesion has interrupted the internal elastic membrane and has partially occluded the lumen of the vessel. A large dissecting aneurysm was observed in the external iliac artery proximal to the origin of the femoral artery just described. That even the small arteries of experimental rats were subject to elastolytic changes is illustrated in Figure 12, in which is shown a mesenteric arteriole with a markedly eroded internal elastic lamina. For purposes of comparison a normal mesenteric artery of comparable size is presented in Figure 13. It should be stated that the elastolytic changes of the type just described were usually masked by stains such as Verhoeff's hematoxylin and Weigert's resorcin-fuchsin. Alteration of elastic fibers were more distinctly demonstrated with H. A. and Mallory's P. A. H.

Demonstrable in the tunica media throughout the abdominal as well as the thoracic portion of the aorta of every experimental animal was an increase both of cellular and extracellular elements. The new cellular elements, again, resemble fibroblasts rather than smooth-muscle cells. Most of the abnormal extracellular material, like that seen in the smaller disease-ridden arteries, stains black with Verhoeff's hematoxylin, light reddish-brown with orcein, pale orange with Mallory's P. A. H., and blue with H. A.;

Fig. 14.—Applied to both aspects of all but the innermost of the elastic laminae of this normal thoracic aorta are short reticular fiber loops, hairpin-like in form. At the apex of each the argyrophilic fiber loops are cemented to the elastic lamina. Longitudinal section, Foot-Menard's reticulum stain and a light counterstain with Harris' hematoxylin; reduced ⅔ from mag. × 500.

Fig. 15.—As seen in this section of an experimental rat's thoracic aorta, the pale-stained elastin "core" of most of the laminae has become nonuniformly attenuated. At the same time the argyrophilic fiber coating appears to have become even denser than in the normal control. Longitudinal section, Foot-Menard's stain and a light counterstain with Harris' hematoxylin; reduced ⅔ from mag. × 500.

Fig. 16.—At a stage of the disease process advanced beyond that represented in Figure 15 it is evident that in place of elastic laminae there now are strata consisting almost entirely of argyrophilic fibers. Longitudinal section, Foot-Menard's stain and a light counterstain with Harris' hematoxylin; reduced ⅔ from mag. × 500.

Fig. 17.—The uniformly wide whitish bands represent the intact elastic laminae of a normal control rat's thoracic aorta. The nonelastic fibrous coating of each lamina appears as fine irregularly wavy dark lines which intervene everywhere between the smooth-muscle cells and the elastic laminae. The high degre of uniformity with rspect to size, shape, and arrangement of all the histologic elements is always an impressive feature of the normal aortic wall. Longitudinal section, Heidenhain's stain; reduced ⅔ from mag. × 500.

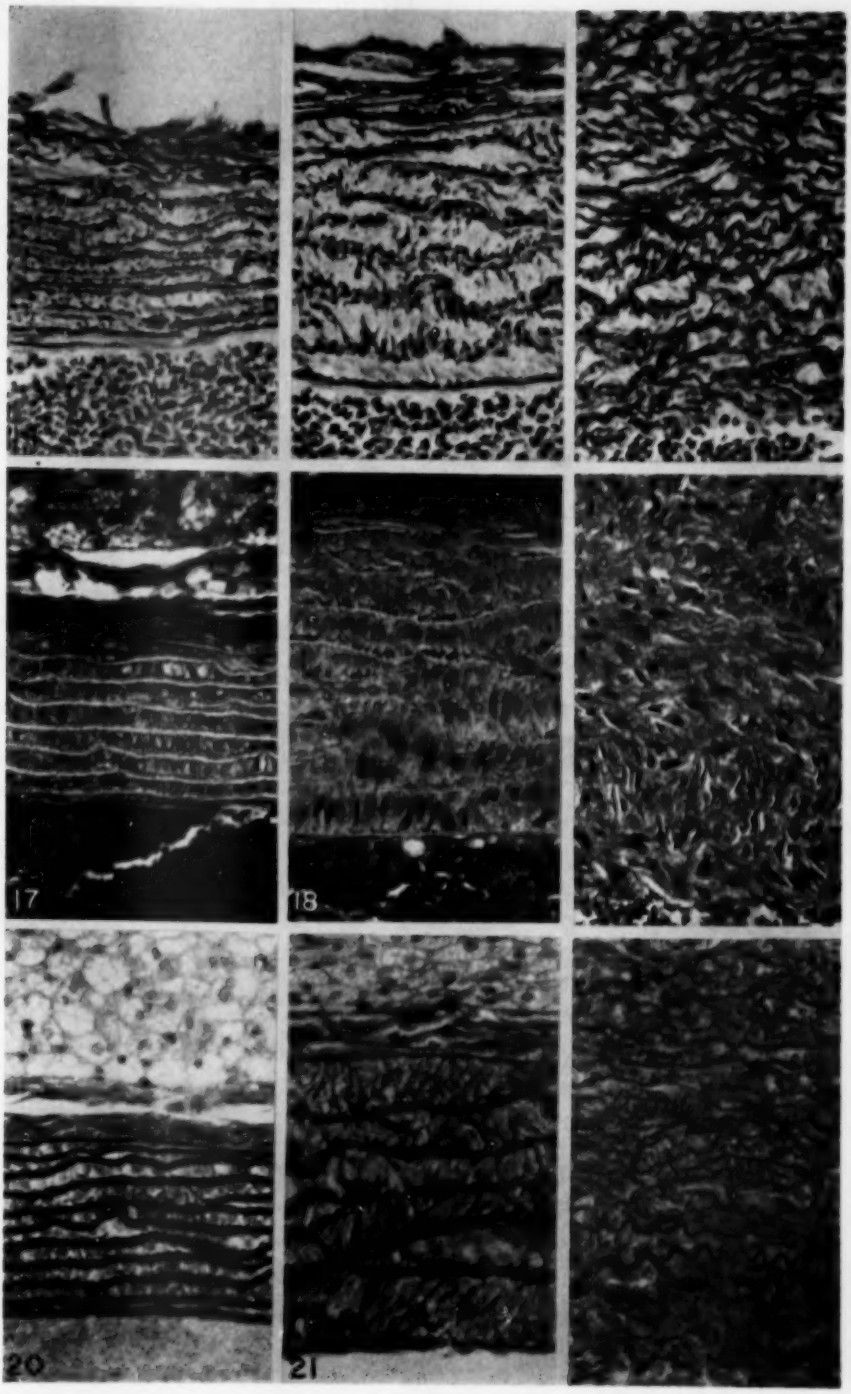
Fig. 18.—Contributing to the chaos of histologic architecture disclosed at moderately far-advanced stages of the disease process such as is seen here are (*a*) the abnormal presence of large pale-stained radially oriented cells, (*b*) a diminution in thickness or complete absence of whitish elastic laminae, and (*c*) an increase in the amount of darkly stained nonelastic laminar coatings. Longitudinal section, Heidenhain's stain; reduced ⅔ from mag. × 500.

Fig. 19.—The replacement of elastic laminae by darkly stained nonelastic fibrous elements is nearly complete in the far-advanced stages of the disease as shown in this figure. Longitudinal section, Heidenhain's stain; reduced ⅔ from mag. × 500.

Fig. 20.—As seen in this specimen of a normal rat's thoracic aorta both the elastic laminae and the coatings of each are intensely stained by the P. A. S. reagent. Longitudinal section, McManus' periodic acid-Schiff stain; reduced ⅔ from mag. × 500.

Fig. 21.—In this aorta of an experimental rat a reduction in intensity of reaction to the P. A. S. reagent was noted. The P. A. S.-positive material is more diffusely distributed than in normal specimens. Longitudinal section, McManus' periodic acid-Schiff stain; reduced ⅔ from mag. × 500.

Fig. 22.—At advanced stages of the disease the thoracic aorta, although rich in reticular fibers, is only weakly responsive to the P. A. S. reagent. Longitudinal section, McManus' periodic acid-Schiff stain; reduced ⅔ from mag. × 500.


17
18
20
21

is argyrophilic, and is either unstained or only very faintly stained with both toluidine blue and the P. A. S. reagent. To illustrate some of the foregoing histopathological findings are photomicrographs of sections through the thoracic portion of the aorta of a normal control rat (Figs. 14, 17, and 20) and of two experimental animals to represent the disease process in moderately advanced (Figs. 15, 18, and 21) and far-advanced stages (Figs. 16, 19, and 22). In the silver-impregnated specimen of the normal rat aorta (Fig. 14) the tightly knit short loops of reticular fibers are closely applied to both aspects of the elastic laminae to form a continuous coating which not only helps to hold together adjacent elastic laminae but also serves as a "spring-like" anchorage for the interlaminar smooth-muscle cells. This same coating stains blue with H. A. (Fig. 17). All intact elastic laminae and the coatings of each were markedly P. A. S.-positive (Fig. 20), a reaction not lost by pretreatment in 5% phenylhydrazine. In the aortae of the experimental rats there is a progressive infiltration of fibroblasts and a gradual loss of the elastic laminae. The argyrophilic coating of each lamina becomes thicker (Fig. 15) and remains as an elaborate feltwork even after the elastic laminae have disappeared (Fig. 16). At those sites where the pathological process was far advanced there often was a very substantial increase in content of collagenous fibers, recognizable on the tinctorial basis of lacking an affinity for silver and yet being stainable with Van Gieson's picrofuchsin mixture. It would appear that as an elastic lamina underwent disintegration its fibrillar investment became increasingly dense owing to the formation of new reticular fibers which eventually would lose their affinity for silver to become mature collagenous fibers. In H. A.-stained sections the abnormally dense feltwork of reticular (and collagenous) fibers is stained blue (Figs. 18 and 19) but is only faintly stained with the P. A. S. reagent (Figs. 21 and 22). The latter dissociation of reticular fibers and P. A. S.-stainable material is particularly noteworthy.

Coarse elastic fibers of uniform diameter were virtually absent from the connective tissue spreads excised from various portions of the body cavities and subcutaneous panniculus of the experimental rats. The deficiency regarding elastic fiber content was nowhere more apparent than in the mesoappendix, in which a dense feltwork of coarse elastic fibers normally has formed by six weeks of age (Fig. 23). The experimental rat's mesoappendix (Fig. 24)

Fig. 23.—Elastic fibers of the mesoappendix of a normal rat 6 weeks of age. The field was selected to represent one of intermediate density with respect to content of elastic fibers. Full-thickness spread, Verhoeff and Van Gieson's stain; reduced ⅔ from mag. × 500.

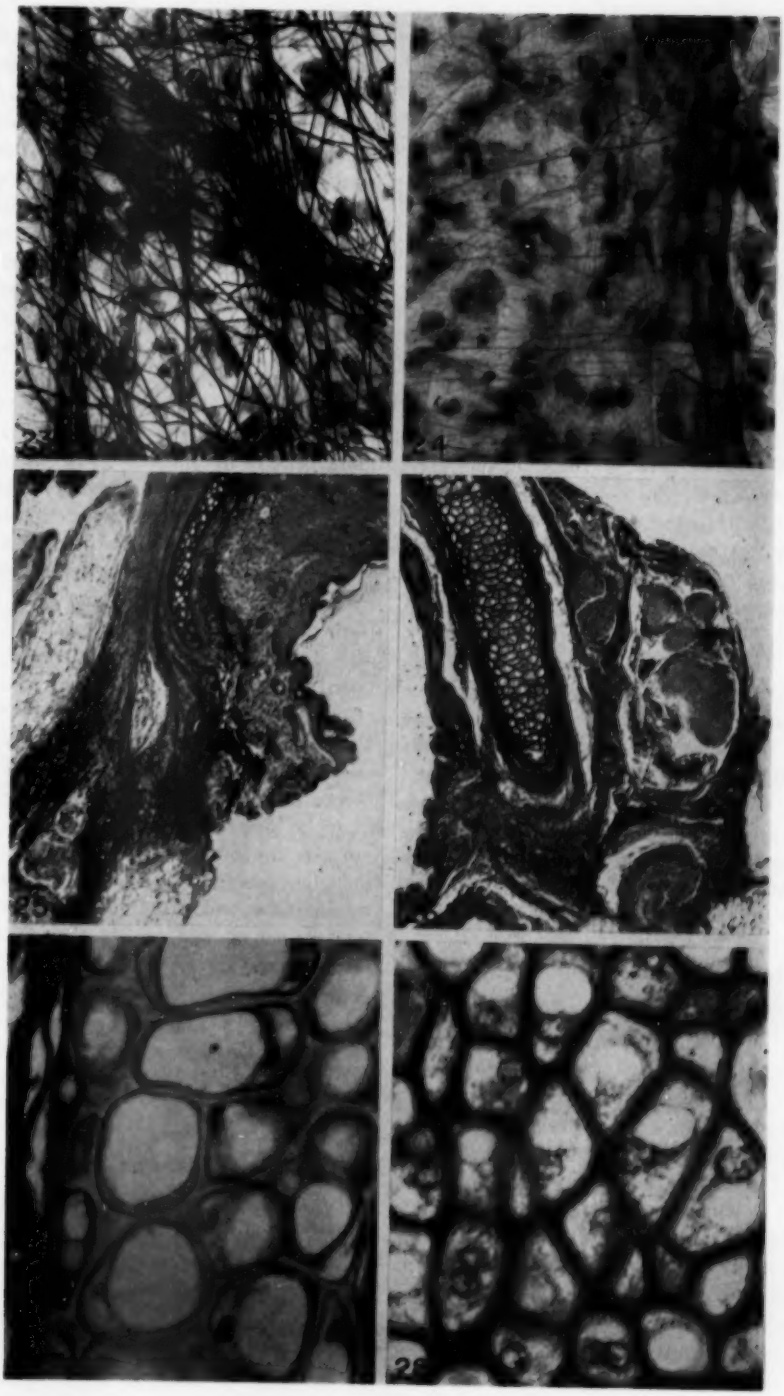
Fig. 24.—In the mesoappendix of an experimental rat 6 weeks of age only fine faintly stained fibrils were seen. Verhoeff and Van Gieson's stain; reduced ⅔ from mag. × 500.

Fig. 25.—In the wall of the external ear canal of a normal control rat 6 weeks of age the black reaction characteristic for elastin is seen in the cartilage matrix and in the dense network of subcutaneous fibers adjacent to the cartilage. Transverse section, Verhoeff and Van Gieson's stain; reduced ⅔ from mag. × 100.

Fig. 26.—This specimen of the external ear canal of an experimental rat 6 weeks of age shows a lack of elastin in the cartilage matrix and an absence of elastic fibers in the adjacent areas of subcutaneous connective tissue. Transverse section, Verhoeff and Van Gieson's stain; reduced ⅔ from mag. × 100.

Fig. 27.—Elastic cartilage of the external ear canal of a normal rat 6 weeks of age. Only a small proportion of the space in the comparatively enormous central lacunae is occupied by the chondrocyte. The nucleus of the latter is usually small, darkly stained, and of crescentic shape. The denser portions of the elastic matrix in this figure are represented as thick whitish bars intervening between the centrally placed lacunae. Transverse section, Heidenhain's stain; reduced ⅔ from mag. × 750.

Fig. 28.—Elastic cartilage of the external ear canal of an experimental rat 6 weeks of age. The cartilage cells which in most instances largely fill the lacunar spaces usually have rather large oval more or less centrally placed nuclei which contain prominent nucleoli. The matrix lacks the thick white bars representing elastic matrix characteristic of normal ear cartilage. Transverse section, Heidenhain's stain; reduced ⅔ from mag. × 750.



contains only very fine fibers, most of which have a weak affinity for the elastic stain (Verhoeff's hematoxylin). No amorphous deposits or elastic fiber coatings of any kind were seen in any of the membranes studied.

The matrix of the cartilage in the wall of the external auditory meatus of experimental animals showed a marked reduction of elastin as revealed by Verhoeff's hematoxylin (Fig. 26) and a weaker metachromatic reaction to toluidine blue and a less intense reaction to the P. A. S. reagent than the corresponding elastic cartilages of normal control animals. The reduction of elastic fiber content of the perichondrium and of the neighboring subcutaneous connective tissue is also readily apparent when the experimental specimen is compared with that of the control shown in Figure 25. As in the case of the intact arterial elastic laminae, the dense bars of elastin seen encircling the lacunae of mature chondrocytes of the normal specimens are highly refractile and appear whitish in color, since they are only faintly stained by the aniline blue (Fig. 27). No such whitish bars are seen in the matrix of the experimental rat's elastic cartilage (Fig. 28). The cartilage cells of the experimental rat's elastic cartilage seemed to have retained certain immature features, such as a highly vacuolated cytoplasm that nearly fills the lacuna and a large oval vesicular nucleus with one or two prominent nucleoli.

Comment

In contradistinction to a number of other studies [1,4-6,8] the present investigation has disclosed evidence of widespread arterial involvement in young rats that had been treated with the L. odoratus factor. The extent to which the arterial system becomes involved is related to the age period during which the animal is exposed to the toxin. To produce elastic fiber alterations elsewhere than in the aorta the toxin must be administered during the first few weeks after birth, when elastogenesis normally is particularly active.[17-21] Rats over 7 weeks of age are not subject to the formation of aortic aneurysms. These findings indicate that the disturbance is the result of inhibition to the formation of elastic fibers rather than the effect of a direct-acting elastolytic agent. Fragmented elastic laminae per se do not necessarily represent the effects of a lytic process. At an early stage in their normal histogenesis, as demonstrated with elastic stains, elastic laminae appear as a row of granules or a series of short fine filaments.[20] It has also been reported that at a very early stage of differentiation, when they have no affinity for orcein or resorcin fuchsin, aortic elastic laminae are argyrophilic.[19,21] In the present study of the aortic lesions of lathyrism it was noted that where the deficiency of elastic laminae was most conspicuous the argyrophilic feltwork was unusually dense, a change accompanied by a diminution in the amount of P. A. S.-positive material. According to Hall, Reed, and Tunbridge [22] elastic laminae are composed of fine fibrils embedded in a dense cement substance. Furthermore, Banga, Bálo, and Szabó [23] have found that this cement substance consists of two different mucoids, one of which, the "sheath" mucoid, may be selectively digested with elastomucase, the other of which is less soluble than the "sheath" mucoid and has been extracted from collagen as well as elastin. An integration of the observations made by Churchill et al.[2] and Ponseti et al.,[5,12] together with those of the present communication, suggests that the specific metabolic defect of lathyrism concerns inhibition of the synthesis of the less soluble mucoid described by Banga et al.

Summary and Conclusions

Widespread arterial involvement with dissecting aneurysms has been produced in rats treated from birth with the Lathyrus odoratus factor. Histologically the arterial lesions are usually characterized by (1) fibroblastic proliferation, (2) partial to complete deficiency of intact elastic laminae, and

(3) increase in the argyrophilic fiber laminar coating. In addition in the far-advanced stages of the pathological process an increase in collagenous fibers and a decrease in periodic acid-Schiff-positive and metachromatic material are evident in walls of all the major arteries. Failure of the formation of elastin was demonstrable in a variety of connective tissue membranes and in the matrix of the external ear canal cartilage, which normally is of the elastic variety.

Department of Anatomy, The Johns Hopkins University School of Medicine, 710 N. Washington St. (5).

REFERENCES

1. Bean, W. B., and Ponseti, I. V.: Dissecting Aneurysm Produced by Diet, Circulation 12:185-192, 1955.

2. Churchill, D. W.; Gelfant S.; Lalich, J. J., and Angevine, D. M.: Alterations in the Polysaccharides and Elastic Fibers in the Aortas of Rats Fed Toxic Lathyrus Factor, Lab. Invest. 4:1-8, 1955.

3. Chang, C. Y.; Witschi, E., and Ponseti, I. V.: Teratogenic Effects of Lathyrus Odoratus Seeds on Development and Regeneration of Vertebrate Limbs, Proc. Soc. Exper. Biol. & Med. 90:45-50, 1955.

4. Ponseti, I. V., and Baird, W. A.: Scoliosis and Dissecting Aneurysm of the Aorta in Rats Fed with Lathyrus Odoratus Seeds, Am. J. Path. 28:1059-1077, 1952.

5. Ponseti, I. V.: Lesions of the Skeleton and of Other Mesodermal Tissues in Rats Fed Sweet-Pea (Lathyrus Odoratus) Seeds, J. Bone & Joint Surg. 36:1031-1058, 1954.

6. Bachhuber, T. E., and Lalich, J. J.: Effect of Sweet Pea Meal on the Rat Aorta, A. M. A. Arch. Path. 59:247-253, 1955.

7. Walker, D. G., and Wirtschafter, Z. T.: Histopathogenesis of Aortic Aneurysms in the Lathyrus-Fed Rat, A. M. A. Arch. Path. 61:125-135, 1956.

8. Lalich, J. J.: Production of Aortic Rupture in Rats Fed Purified Diets and Beta-Aminopropionitrile, A. M. A. Arch. Path. 61:520-524, 1956.

9. Stamler, F. W.: Reproduction in Rats Fed Lathyrus Peas or Aminonitriles, Proc. Soc. Exper. Biol. & Med. 90:294-298, 1955.

10. Pyörälä, K.; Seppälä, T., and Punsar, S.: Aorten- und Knochenveränderungen beim Rattenfötus verursacht durch Fütterung von Lathyrus-Samen an Muttertiere, Acta path. et microbiol. scandinav. 38:273-284, 1956.

11. Walker, D. G., and Wirtschafter, Z. T.: Resorption of Embryos in Rats on Lathyrus Odoratus Diet, J. Nutrition 58:147-160, 1956.

12. Ponseti, I. V.; Wawzonek, S.; Shepard, R. S.; Evans, T. C., and Stearns, G.: Further Studies on Lathyrism in the Rat, Proc. Soc. Exper. Biol. & Med. 92:366-369, 1956.

13. Baló, J.: Die mit Ammoniumhydroxydvergiftung erzeugbare experimentelle Arteriosclerose, Frankfurt. Ztschr. Path. 52:205-220, 1938.

14. Baló, J.: Die Wirkung des Thyroxins auf die Arterien, Beitr. path. Anat. 102:341-360, 1939.

15. Lillie, R. D.: Histopathologic Technic and Practical Histochemistry, Ed. 2, New York, The Blakiston Company, 1954.

16. Gillman, T.; Penn, J.; Bronks, D., and Roux, M.: Abnormal Elastic Fibers: Appearance in Cutaneous Carcinoma, Irradiation Injuries, and Arterial and Other Degenerative Connective Tissue Lesions in Man, A. M. A. Arch. Path. 59:733-749, 1955.

17. Loisel, G.: Formation et évolution des éléments du tissu élastique, J. l'anat. et physiol. 33: 129-197, 1897.

18. Mall, F. P.: On the Development of the Connective Tissues from the Connective Tissue Syncytium, Am. J. Anat. 1:329-365, 1901-1902.

19. de Kervily, M.: Les granulations des élastoblastes et les premiers stades de developpement des fibres élastiques révélés par l'impregnation à l'argent, Compt. rend. Soc. biol. 90:1022-1023, 1924.

20. Krompecher, S.: Die Entwicklung der elastischen Elemente der Arterienwand, Ztschr. Anat. 85:704-723, 1927.

21. Rainville, E.: Stages in the Life History of Elastic and Reticular Fibers in the Aortae of Timed Chick and Mouse Material, Master's Thesis, Mount Holyoke College, South Hadley, Mass., 1946.

22. Hall, D. A.; Reed, R., and Tunbridge, R. E.: Electron Microscope Studies of Elastic Tissue, Exper. Cell. Res. 8:35-48, 1955.

23. Banga, I.; Baló, J., and Szabó, D.: Metacollagen as the Apparent Elastin, J. Gerontol. 11:242-250, 1956.

Myocarditis in Infancy

OTTO SAPHIR, M.D., and NATHAN A. COHEN, M.D., Chicago

In recent years there has been an increasing awareness of nonspecific myocarditis in infants, and there seems to have been a distinct increase in the number of reported instances of isolated and viral myocarditis. According to Conlin and Mantz,[1] only 26 such cases had been reported up to 1953. We have found since 1953 reports of 29 additional autopsied cases of myocarditis in infants in the American literature and 39 in the foreign literature. Since 1950 there have been a total of 38 and 41 reports, respectively.[2-28] Stoeber,[29] in 1952, reported 140 cases, 68 of them with microscopic examinations, which she had collected over a seven-year period. There probably are many more unpublished and many more overlooked cases. In a review in 1944, Saphir et al.[30] pointed out that many cases had not been reported as myocarditis but had been included only as incidental findings in reports of the mythical "status thymicolymphaticus." Though Gormsen[11] stated that "isolated diffuse myocarditis is a rare cause of death in infants," Saphir et al.,[15,30] Bowden,[19] van Creveld et al.,[14,17] and Jevett et al.[2] have stressed its importance.

The increasing reports of nonspecific myocarditis may be due to a greater interest in this disease and, hence, better diagnosis and recognition, or they may be the result of an increasing incidence of myocarditis. From a number of more recent reports stressing even epidemic proportions of the disease occurring in different sections of a large community,[29] from reports of small epidemics in Holland and South Africa,[25,26,31] from the occasional finding of neutralizing antibodies to certain viruses in the mothers of infants dying from the disease,[23,25] as well as in the sera of surviving children,[25] and, finally, from the actual recovery of viral agents from the victims of this disease[2,23,25,26,32] it would seem that virus myocarditis in infants is more often encountered now than ever before.

We have been interested in myocarditis over a number of years but up to 1952 had been able to find in our autopsy material only 11 instances of myocarditis in infants. Since that time we have encountered five additional infants dying from myocarditis. One of these was brought to our observation from the outside. From evidence to be presented subsequently, it seems that none of these myocarditides fall into the classification of isolated myocarditis, i. e., myocarditis of unknown origin occurring in the absence of any disease entity in the wake of which myocarditis is known to occur. Although no viral studies could be made in any of these cases, a viral origin of the myocarditis, as will be shown, seems most likely.

In the following report a short clinical history and autopsy findings of the five instances of myocarditis in infants will be given, with a discussion of their classification and morphologic characteristics and their probable origin.

Report of Cases

CASE 1.—An 11-day-old Negro girl was delivered at term by repeat cesarean section. The mother was Rh-positive, Wassermann-negative, and had an uneventful pregnancy. The birth weight was 2240 gm. At the age of 7 days the infant had several episodes of vomiting of bright red blood. At this time she was afebrile and pale

Submitted for publication April 26, 1957.

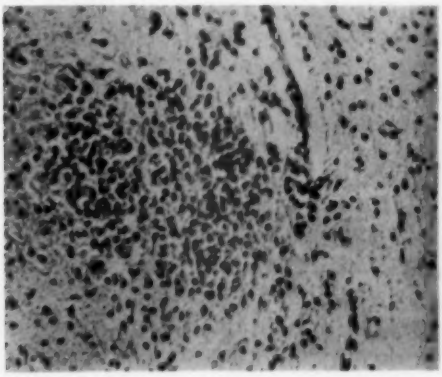
From the Department of Pathology, Michael Reese Hospital.

This research was supported in part by the Lebold Memorial Fund and in part by the Byron C. Sharpe Fund.

and had an enlarged liver and spleen. There were no petechiae or purpura. Originally 18 gm. per 100 cc. at the onset of the illness, the hemoglobin fell to 9 gm. after three days. The white blood cell count was 22,000. Urinalysis disclosed 2+ albumin and a few granular and hyaline casts. There were 58,000 platelets, the bleeding and clotting times were 4½ and 5¼ minutes, respectively, and the prothrombin time was 23 seconds (normal, 12 seconds). Nose and throat cultures disclosed coagulase-negative Staphylococcus albus and a few subtilis organisms. A blood culture remained sterile. Diagnoses of thrombocytopenic purpura and amegakaryocytic thrombocytopenia were considered. The infant continued to do poorly, developing dyspnea, irregular bradycardia, and hypothermia. She died suddenly on the fourth day of illness.

Autopsy (Only the pertinent findings are presented.).—The lungs, liver, and spleen were grossly hyperemic. There was an interstitial pneumonia and a minimal acute bronchopneumonia. Histologically, many liver cells in the midzonal regions were necrotic, with only the periportal and centrilobular regions spared. In scattered areas there was an infiltrate of polymorphonuclear leukocytes and histiocytes. There was bile stasis. In the cerebrum and in the cerebellum there were microscopic foci of necrosis, with an infiltration of small round cells and histiocytes. There was a perivascular cuffing with small round cells and histiocytes. The bone marrow was normal.

Fig. 1 (Case 1).—Cerebellum. Note the more or less circumscribed infiltration of round cells and very few polymorphonuclear leukocytes. Hematoxylin and eosin; reduced approximately ¼ from mag. × 200.



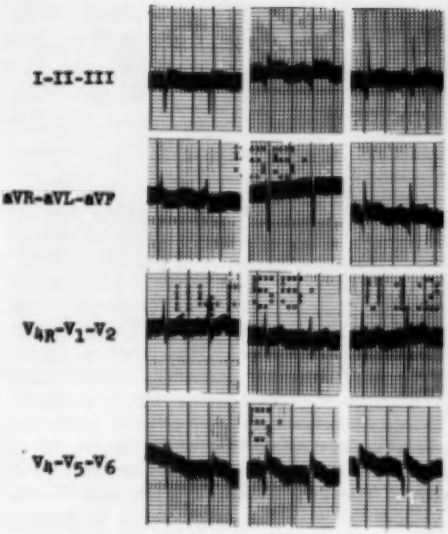


Fig. 2 (Case 2).Twelve-lead electrocardiogram (see text).

There were histiocyte-infiltrated necrotic foci in the pancreas, and numerous polymorphonuclear leukocytes were scattered throughout the thymus.

Heart: The heart was enlarged and slightly dilated. No gross abnormalities of the myocardium were noted. Microscopically, there was a moderate spreading apart of muscle bundles. The muscle fibers were intact except in a few scattered minute focal areas, where there was dissolution of the sarcoplasm and fragmentation of the sarcolemma sheaths. The myocardial nuclei in these foci were hyperchromatic, pyknotic, or fragmented. Immediately adjacent muscle fibers had sarcoplasm with a smudgy appearance and nuclei with somewhat eccentrically located chromatin material. There was a diffuse interstitial infiltrate of lymphocytes, histiocytes, and a few polymorphonuclear leukocytes and plasma cells. The cellular infiltrate was slightly more marked in the necrotic foci. There was a moderate perivascular infiltrate of similar cells. Capillaries were moderately dilated and filled with red blood corpuscles. There were varying concentrations of inflammatory cells within an edematous pericardium, with the

proportion of polymorphonuclear leukocytes and plasma cells being greater than in the myocardium. There were a few foci of inflammatory cells in the endocardium. Inclusion bodies were not found in either hematoxylin-and-eosin- or Giemsa-stained sections.

Case 2.—An 11-day-old girl apparently was well until the age of 9 days, when she refused feedings and developed tachypnea. Subsequently, respirations became labored, and she was hospitalized. The temperature was 99.6 F; pulse, 180, and respirations, 96. The infant was in marked respiratory distress, but no rales were heard. There were no cardiac murmurs. The liver was markedly enlarged. Laboratory work disclosed a hemoglobin of 18 gm. per 100 cc., white blood corpuscle count of 10,600, and a serum urea nitrogen level of 53 mg. per 100 cc. An electrocardiogram (Fig. 2) revealed a sinus rhythm, prominent Q waves, and elevated ST segments in Leads II, III, aV_F, and V_{4-6}, on the basis of which an anomalous origin of one of the coronary arteries was suspected.* The infant developed cyanosis and died on the second hospital day.

Autopsy (Only the pertinent findings are given.).—There were slight ascites, hydrothorax, and hydropericardium.

There was an interstitial pneumonia and bronchopneumonia. The abdominal viscera were hyperemic, and the liver was enlarged. In the liver there were scattered focal areas of centrilobular and peripheral necrosis, with associated hemorrhage and slight polymorphonuclear and mononuclear cellular infiltration. The medullary portions of the thymus contained scattered eosinophils and polymorphonuclear leukocytes. The brain was essentially normal.

Heart: The heart was totally enlarged, with the right side being three times the size of the left. The ductus arteriosus and the foramen ovale were patent. The coronary artery distribtuion was normal. There were a few petechiae involving the septal surface of the left ventricle. The myocardium was pale and flabby, and there were pale, yellow-gray areas. Microscopically, muscle fibers and bundles were moderately spread apart. In sections of left ventricle approximately one-third of the myocardial fibers were altered. In many diffuse areas striations were lost and the sarcoplasm was changed to an amorphous- to smudgy-appearing eosinophilic material. There was a varying degree of vacuolization of the cytoplasm. In the midst of these altered areas, some groups of myocardial fibers had hyperchromatic to pyknotic to fragmented nuclei. A pleomorphic cellular exudate was scattered throughout the myocardium, with an increased affinity for the areas of necrosis. There were varying proportions of mononuclear cells and polymorphonuclear leukocytes, with the former generally predominating. A rare eosinophil was present. The perivascular cellular infiltrate was proportionate to the infiltrate elsewhere in the myocardium. The capillaries were dilated and engorged with red blood corpuscles. Within the epicardium there were aggregates of closely packed mononuclear cells, generally resembling lymphocytes. Inflammatory cells were present in small numbers in the subendocardial connective tissue, and there was a small mural thrombus. No inclusion bodies were found in hematoxylin-and-eosin- or Giemsa-stained sections.

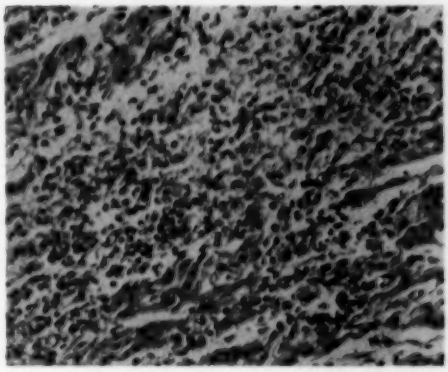


Fig. 3 (Case 2).—Note the spreading of muscle bundles, the degeneration and necrosis of muscle fibers, and the marked infiltration of monocytic cells and a few polymorphonuclear leukocytes. Hematoxylin and eosin; reduced 1/5 from mag. ×200.

* This and Case 4 are included in a separate electrocardiographic study on coronary patterns in infancy, to be published by Drs. P. Dominguez and A. Pick.

Fig. 4 (Case 3).—Note the severe infiltration of mainly round cells and the necrosis of individual muscle fibers. Hematoxylin and eosin; reduced 1/5 from mag. × 200.

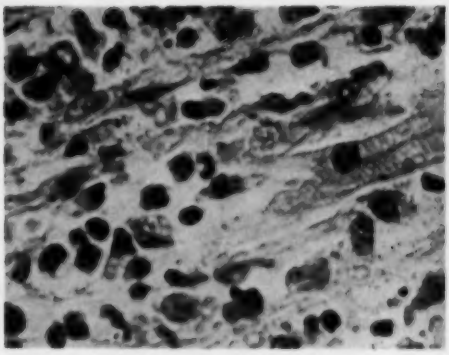


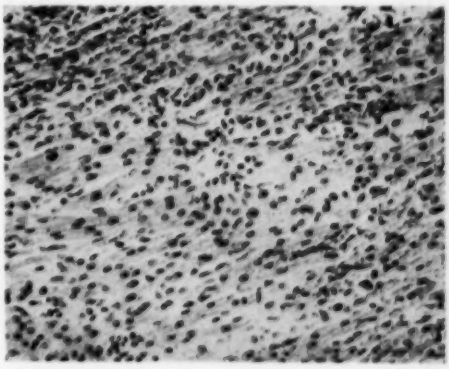
Fig. 5 (Case 3).—Note the destruction of muscle fibers and the infiltration by monocytes. Hematoxylin and eosin; reduced approximately ¼ from mag. × 450.

Case 3.†—A baby girl had slight and temporary respiratory difficulties following delivery at home. No abnormalities were noted until the seventh day of life, when cyanosis appeared. She died approximately six hours after hospitalization. The infant had been breast-fed. Two or three days before the infant's death the mother had a respiratory infection and a unilateral mastitis and was treated with penicillin.

Autopsy (The heart, lungs, liver, kidneys, suprarenals, and thymus were examined microscopically. Only the pertinent findings are given.).—In the lungs there was severe edema and acute passive hyperemia, with foci of hemorrhage. Scattered histiocytes and a few polymorphonuclear leukocytes were present in the periportal spaces of the liver and in the connective tissue septi of the thymus.

Heart: There were varying degrees of edema. In most of the sections taken from the right and left ventricles there were scattered diffuse areas of interstitial and focal cellular exudate, with polymorphonuclear leukocytes predominating. There were moderate numbers of histiocytes and scattered lymphocytes. In the midst of the areas with the most intense inflammation, the muscle fibers were necrotic. Adjacent fibers were degenerated, showing varying degrees of eosinophilic smudging of the sarcoplasm, vacuolization, and hyperchromatic and pyknotic nuclei. In one section there were degenerative changes without associated inflammatory infiltrates. Diffuse hemorrhage was present in one area of inflammation and focal necrosis. At the periphery of, or adjacent to, some of the inflamed areas, there were strands of fibroblasts and young connective tissue, in some instances surrounding fragments of intact but hypertrophied muscle fibers. Nerve ganglia contained well-preserved ganglion cells; however, the matrix was edematous, and a few scattered distorted granulocytes were present. A section taken from the region of the sinoauricular node disclosed edema, polymorphonuclear leukocytes, and

Fig. 6 (Case 3).—Note the diffuse infiltration of principally polymorphonuclear leukocytes. There are also degenerative changes of individual muscle fibers. Hematoxylin and eosin; reduced approximately ¼ from mag. × 200.



† Dr. A. Vass, Mount Vernon, Ill., gave permission for the use of this case.

chronic inflammatory cells. Blood vessels and valve leaflets showed no striking histopathologic changes. Six of the eighteen sections studied showed no changes other than variable degrees of edema.

CASE 4.—A 9-month-old boy died two hours after admission. He had had cough and coryza for one week and became dyspneic one day prior to admission. Physical examination revealed pallor, lethargy, dyspnea, hyperemic throat, and a heart rate of 270. The liver was enlarged. In the electrocardiogram a regular supraventricular tachycardia was found of a rate of 214, with upright P waves superimposed on ST-T segments which showed a "monophasic" deformation so that, as in Case 2, an anomalous origin of one of the coronary arteries was suggested.

Autopsy (Only the pertinent findings are given.).—There was acute passive hyperemia of the lungs and liver and a terminal bronchopneumonia. The brain could not be examined.

Heart: The heart was of normal size. The distribution of the cornonary arteries was normal. The myocardium was flabby, and the posterior wall of the left ventricle appeared pale. Microscopically, the individual muscle fibers were fragmented. In scattered large areas the sarcoplasm was altered to an intensely staining homogeneous eosinophilic material. Some fibers were vacuolated. Within and adjacent to these areas were foci where both sarcoplasm and nuclei could not be identified, and an amorphous eosinophilic material remained in partially fragmented sarcolemma sheaths. Nuclei in neighboring areas were pyknotic. There were interstitial and focal aggregates of inflammatory cells, with a slightly greater concentration in the necrotic areas. There were lymphocytes, polymorphonuclear leukocytes, and plasma cells in varying proportions. The capillaries were dilated and engorged with blood. A few scattered inflammatory cells were present in the epicardium. Inclusion bodies were not found in hematoxylin-and-eosin- or Giemsa-stained sections.

CASE 5.—A 12-month-old white girl had fever and vomiting two days prior to admission. She became increasingly irritable and dyspneic and was admitted after failure to respond to penicillin and sulfonamides. On admission, the temperature was 102 F; pulse, 192; respirations, 90, and blood pressure, 100/? mm. of Hg. The infant was pale, dyspneic, and cyanotic. The lung fields were clear. The liver was not palpable, and there was no edema. The WBC was 17,350, and the Hb., 11.5 gm. per 100 cc. An electrocardiogram disclosed a sinus tachycardia of 214, large P waves, and nonspecific T wave flattening in all leads. Enterococcus was later cultured from a venous blood specimen. Treatment consisted of streptomycin, penicillin, and oxygen. The infant became more dyspneic and cyanotic. A diagnosis of myocarditis was entertained, but the patient died two and one-half hours after admission, before a digitalis preparation could be administered.

Autopsy (Only the pertinent findings are presented.).—There was slight pitting edema, right hydrothorax, and ascites. There was slight terminal bronchopneumonia and acute passive hyperemia of the viscera. The brain was edematous. Other than in the heart and lungs, there were no foci of inflammation.

Heart: The heart was markedly enlarged, especially the left ventricle, and weighed 80 gm., instead of a normal weight of about 44 gm. The myocardium was flabby and had numerous small ill-defined gray discolored areas. Microscopic study disclosed muscle fibers and bundles to be slightly separated by edematous interstitial tissue. Except for a few scattered minute focal areas associated with a marked inflammatory cellular infiltrate, the fibers were intact, and the striations were apparent. In all sections there were diffuse cellular interstitial infiltrations and scattered focal aggregates of cells. The predominating cells were lymphocytes, but a few scattered histiocytes and polymorphonuclear leukocytes were present. In the focal areas mentioned above, some muscle fibers were fragmented; others had an amorphous eosinophilic material replacing the usual sarcoplasm structure. Some of these fibers appeared to be definitely necrotic. A few of the immediately adjacent muscle fibers had no striations. There was no definite relationship of the cellular exudate to blood vessels. Capillaries were dilated and engorged with red blood corpuscles. The epi-

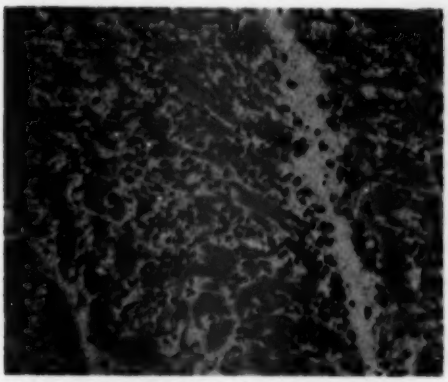


Fig. 7 (Case 5).—Note necrosis of muscle fibers and diffuse infiltration of lymphocytes and few polymorphonuclear leukocytes. Iron hematoxylin and eosin; reduced ⅓ from mag. × 200.

cardium was edematous, and in several instances there were minute foci of inflammatory cells contiguous with the myocardial exudate. There were minute foci of lymphocytes in the endocardium.

Summary of Cases

Five infants, two of whom were 7 days old; one, 11 days; one, 9 months, and one, 12 months, died after a very short illness. Evidence of myocardial changes was found clinically or electrocardiographically three times. In two instances the electrocardiographic changes were first interpreted as being the result of abnormal origin of the left coronary artery from the pulmonary artery. At autopsy in all infants myocarditis was disclosed which was associated with necrosis of varying degrees of groups or of isolated muscle fibers.

Comment

Of the five patients presented, three died within the first two weeks of neonatal life. A study of 90 cases [1-28,33,34] of myocarditis in infants reported since 1948 showed that 47% of these infants were less than 6 months of age, and 71% under 1 year of age. Only 12 of these children died after the age of 18 months. Of the infants less than six months of age, 28 died in their first month. The accompanying graph (Fig. 8) illustrates the decreasing incidence of fatal nonspecific myocarditis with increasing age and brings to mind a mirror image of an infantile antibody-response curve. A possible explanation lies in a fault of antibody response, either the lack of transplacental antibodies or an inadequate active antibody production.

Myocarditis in infancy generally has a rapid fulminating course. Though Javett et al.[2] described a definite biphasic short

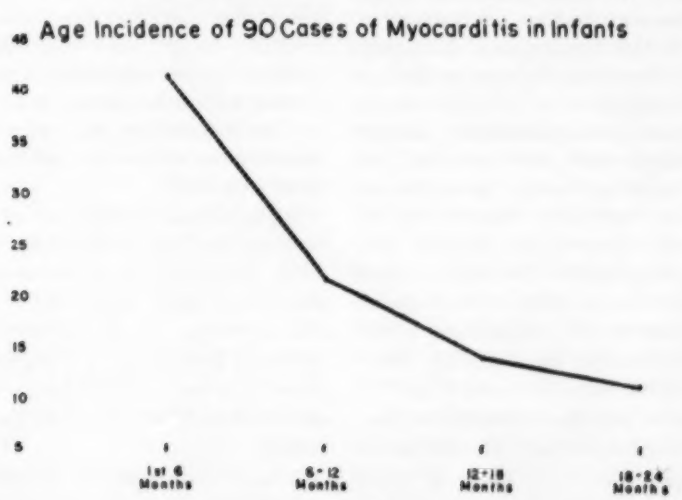


Figure 8

course of myocarditis associated with Coxsackie B virus, van Creveld and De Jager [14,26] reported a progressive monophasic course with Coxsackie B myocarditis. This seems to be generally true of most cases of "nonspecific" myocarditis. While there are sometimes no prodromata, coryza, listlessness or restlessness, or vomiting and diarrhea often have been noted. Once signs of respiratory distress appear, the infants die in a matter of minutes to several days. However, there are findings pointing to involvement of the heart, and electrocardiograms almost always indicate myocardial damage. In spite of marked respiratory distress, there may be minimal pulmonary findings. Cyanosis is a frequent preterminal event. The tachycardia is out of proportion to the low-grade fever often present. Gallop rhythm is a frequent finding.[16,19] There is often an enlargement of the liver and sometimes of the spleen. Dependent edema has been noted infrequently, since most of these patients are in the prewalking age and possibly because sacral edema is not suspected. There is no ready explanation for the prolonged prothrombin time and hematemesis present in one case of the series presented by van Creveld et al.[17] and in Case 1 of our series. A hepatitis was present in the latter but absent in the former. Laboratory findings are nonspecific. It is possible that transaminase determination may point to the myocardial damage present. Rosenbaum et al.[16] emphasized the almost constant roentgenographic findings of an enlarged heart and described the electrocardiographic changes generally indicating left ventricular hypertrophy, T wave changes of myocardial damage, and, sometimes, arrhythmias. The same authors described the clinical differential diagnosis between nonspecific myocarditis and glycogen-storage disease of the heart, an "aberrant" left coronary artery (Cases 2 and 4), medial necrosis of the coronary arteries, and subendocardial fibrosis (fibroelastosis).

At necropsy there are signs of heart failure. The heart is almost always enlarged, and the chambers generally are dilated. Frequently there is hydrothorax and less often ascites. The liver and spleen usually are hyperemic and enlarged. The lungs are hyperemic and sometimes edematous. Gross examination of the myocardium discloses a flabby, often pale-spotted, muscle with indistinct cut surfaces. Microscopically, there is interstitial diffuse or a focal cellular infiltrate, with some edema, with muscle fiber degeneration, and with focal areas of necrosis. We have been unable to find a constant or specific cellular response or histologic pattern. Though lymphocytes or plasma cells generally predominate, there may be varying numbers of polymorphonuclear leukocytes, mononuclear cells, eosinophils, or histiocytes. Sometimes mononuclear cells and histiocytes predominate and suggest an older age of the myocarditis. Granulomatous infiltrates have been described in this age group, but are very rare.[4,10,13] Javett et al.,[2] using the Giemsa stain, found spherical basophilic granules resembling inclusion bodies in the cytoplasm of some histiocytes. Drennan [9] concluded that the inflammatory-cell infiltrate followed myocardial-cell degeneration and necrosis. Javett et al.[2] believed that the cellular infiltrate preceded the muscle-cell changes. In our material we found muscle-fiber degeneration and necrosis generally to be associated with cellular exudates; many nonnecrotic areas were infiltrated with inflammatory cells. However, in Case 3 there was an area of myocardial degeneration without an associated inflammatory exudate.

Slight cellular infiltration of the endocardium and the epicardium is not unusual. In Case 2 there was a small mural thrombus. Saphir and Field [15] found several instances of thromboemboli in the coronary, pulmonary, and cerebral arteries in necropsy material of infantile myocarditis and attributed them to small cardiac mural thrombi.

Isolated myocarditis, with the synonyms of idiopathic, interstitial, or Fiedler's myo-

carditis, is defined as myocarditis with relatively little or no endocardial and epicardial inflammatory changes and without definite associated contributing factors. There are, however, reported instances of isolated myocarditis where full necropsy descriptions have been recorded, especially recently, and associated changes have been noted in several organs, foremost in the lungs, liver, and brain. In reviewing the literature and their cases, Tedeschi and Stevenson [35] and later Trossman and Curtiss [12] were impressed by the association of myocarditis and interstitial pneumonia. They concluded that myocarditis actually may be part of a generalized process. Offhand, it would seem that the myocarditis in these cases was not isolated myocarditis but, because of the presence of the interstitial pneumonia, was of viral origin. It may well be that other cases reported as isolated myocarditis may be either secondary to an interstitial pneumonia and be of viral origin or may be a part of a generalized systemic disease, as the myocarditis reported by Saphir and Amromin.[36] On the other hand, interstitial pneumonia is a common finding in autopsies of the newborn; the association of interstitial pneumonia and myocarditis may be only fortuitous, or it may point to a possible virus origin of the associated myocarditis.

In two of our cases (Cases 1 and 2), besides a marked acute passive hyperemia of the liver, there was definite evidence of hepatitis. In Case 3 there were scattered periportal infiltrates of histiocytes and polymorphonuclear leukocytes. The hepatitis in these cases perhaps may point to a possible virus origin of the myocarditis. Hepatitis was noted by van Creveld et al.[14,17,26] in 5 out of 13 cases. Also, Javett et al.[2] noted hepatitis in some of their cases. Wood [37] and Saphir et al.[38] have described myocarditis in association with epidemic hepatitis.

Encephalitis or meningoencephalitis, most likely of viral origin, is found infrequently with myocarditis. Case 2 of our series had an encephalitis. Javett et al.[2] found similar lesions in one out of five autopsied cases; van Creveld et al.[14,26] in six cases, and Kibrick and Benirschke,[23] in one case. All of these were associated with Coxsackie B virus disease. It is interesting to note that in the cases of Coxsackie myocarditis with complete autopsy reports there was hepatitis associated with encephalitis in five cases, and in only three patients was there myocarditis and encephalitis without hepatitis.[2, 14,23,26] Myocarditis with encephalitis in man has been reported as caused by the encephalomyocarditis virus.[39,40] Even though viruses have been recovered infrequently from the heart, it seems most likely that all these myocarditides associated with known virus diseases are also of viral origin.

As stated above, isolated, or Fiedler's, myocarditis is defined as inflammation of the myocardium of unknown origin in the absence of a noteworthy involvement of either the pericardium or endocardium and in the absence of any disease in the course of which myocarditis is known to occur. From this definition it is immediately evident that the more that is known about various causes of disease and means of spread to the myocardium, the more often lesions first diagnosed as isolated myocarditis will be reclassified under different headings. Thus, myocarditis as occurring in instances of hypersensitivity is not classed as isolated myocarditis. This is also true for myocarditis in parasitic diseases such as trichinosis, where the encysted parasite is not demonstrable in the myocardium. Experimental work, using various bacteria where at autopsy myocarditis is found but no organisms can be discovered in the myocardium and where the myocarditis is explained on a toxic basis,[41,42] cannot be regarded as proof of experimental production of isolated myocarditis.

The difficulties in distinguishing isolated myocarditis from viral myocarditis are especially marked. In both instances the commonly applied methods for discerning a causative agent give negative results. It

is usually, and most likely, correctly assumed that nonsuppurative myocarditis as seen in instances of known and recognized virus diseases are also caused by viruses. In some of these myocarditides the virus actually has been discovered in the myocardium, as in poliomyelitis, Coxsackie virus disease, and influenzal disease. However, in routine autopsies virus studies usually are not done. Besides, myocarditis is often not recognized grossly, and by the time histologic sections are available it is too late to resort to virus studies. In short, there are apparently a number of instances of virus myocarditis which are diagnosed as such principally because they are found in patients with a known virus disease. Yet, if it is not known that these patients had a virus disease or if a viral origin of the disease is not recognized, the accompanying myocarditis may well be classified incorrectly as isolated or Fiedler's myocarditis. This dilemma is especially pertinent in instances of myocarditis in infants. In the Coxsackie virus infection the disease is not severe and may simulate an upper respiratory infection or pleuritis. The virus may be transmitted by pregnant women to the fetus, and epidemics in nurseries have been reported.[2] Such myocarditis in the absence of virus studies may at first well be classified incorrectly as isolated myocarditis.

The question immediately arises as to whether or not isolated myocarditis is not also of viral origin. This question has been raised repeatedly in the past and was particularly pertinent when the encephalomyocarditis virus was discovered.[43] It again was raised more recently by Stoeber,[29] who investigated a number of instances of myocarditis which occurred in epidemic form in Munich, Germany. The spread of the disease in certain communities suggested a viral origin. Stoeber and other investigators stressed that isolated, or Fiedler's, myocarditis involves principally the interstitial tissue of the myocardium. As a matter of fact, it had been originally termed "interstitial myocarditis." Involvement of muscle fibers is rare. However, in virus myocarditis, while much of the exudate is located in the interstitial tissue, degeneration and necrosis of isolated, or groups of, muscle fibers invariably occurs. We have seen this repeatedly in myocarditis in poliomyelitis, in encephalomyocarditis, and in myocarditis in epidemic hepatitis. The necrosis of individual muscle fibers, which may well form the domicile of the virus, seems to us so characteristic that we believe it is sufficiently important to be used as differential diagnostic means between isolated and viral myocarditis. The necrosis involves only isolated muscle fibers or small groups of fibers and is not as massive as seen in diphtheritic myocarditis. The necrotic muscle fibers also do not give rise to a granulomatous reaction as seen in some instances of isolated myocarditis and experimental myocarditis.[44] The type of cellular infiltrate in isolated or in viral myocarditis is not sufficiently distinctive to permit a differential diagnosis.

From the foregoing and from the finding of outspoken foci of necrosis in the myocardium in our cases, it appears that the myocarditis was the result of a virus infection. In our cases, unfortunately, viral studies could not be made. The viral origin can be surmised because of the similarity of some of the findings in our cases to those of Coxsackie myocarditis reported by Gear et al.,[25] van Creveld and De Jager,[26] Verlinde et al.,[32] and Kibrick and Benirschke [23] and because of the histologic findings, of which necrosis of isolated muscle fibers seems most characteristic. It seems most likely that the Coxsackie virus was responsible for the disease, at least in our weeks-old infants. The Coxsackie virus is the only virus we are aware of which has been found in newborn infants to cause myocarditis. Microscopically, the myocarditis in these infants definitely is older than the age of the infants and probably was the result of an intrauterine infection, as had been reported to occur in some of the cases of Coxsackie virus myocarditis.

Summary

The literature on isolated and virus myocarditis in infants and children is reviewed, and the clinical and pathologic findings are outlined. Five cases of myocarditis in infancy are recorded. Although no virus studies could be made, it is most likely that the myocarditis was of viral origin. In three of these infants dying shortly after birth it appeared that the myocarditis was caused by a virus transmitted through the placenta, most likely belonging to the group of Coxsackie viruses. An attempt is made to differentiate isolated (Fiedler's) myocarditis and viral myocarditis. Isolated myocarditis is predominantly an interstitial myocarditis. In virus myocarditis, necrosis of isolated muscle fibers seems to be characteristic. To judge from reported instances and our experience, viral and isolated myocarditis seem to occur more frequently during the last three years than ever before.

Medical Research Institute, Department of Pathology, Michael Reese Hospital, 29th St. & Ellis Ave. (16).

REFERENCES

1. Conlin, G. J., Jr., and Mantz, F. A., Jr.: Isolated Myocarditis in Infants, J. Pediat. 42:414, 1953.

2. Javett, S. N.; Heymann, S.; Mundel, B.; Pepler, W. J.; Lurie, H. I.; Gear, J.; Measroch, V., and Kirsch, Z.: Myocarditis in the Newborn Infant: A Study of an Outbreak Associated with Coxsackie Group B Virus Infection in a Maternity Home in Johannesburg, J. Pediat. 48:1, 1956.

3. Lide, T. N.: Symposium on Myocarditis: Isolated Myocarditis in Infants and Children; Report of 5 Cases, North Carolina M. J. 15:6, 1954.

4. McGreane, N. A.: Fiedler's Myocarditis at Age of 2 Months, Wisconsin M. J. 53:582, 1954.

5. Verger, Pery, and Gatineau: Interstitial Myocarditis and Acute Primary Asystolia in Infants, J. méd. Bordeaux 132:25, 1955.

6. Bastrup-Madsen, P.: Isolated Interstitial Myocarditis of Unknown Etiology: Three Cases in Infants, Acta pædiat. 40:85, 1951.

7. Hackel, D. B.: Myocarditis in Association with Varicella, Am. J. Path. 29:369, 1953.

8. Williams, H.; O'Reilly, R. N., and Williams, A.: Fourteen Cases of Idiopathic Myocarditis in Infants and Children, Arch. Dis. Childhood 28: 271, 1953.

9. Drennan, J. M.: Acute Isolated Myocarditis in Newborn Infants, Arch. Dis. Childhood 28:288, 1953.

10. Goldberg, J. M.: Myocarditis of Giant-Cell Type in an Infant, Am. J. Clin. Path. 25:510, 1955.

11. Gormsen, H.: Sudden, Unexpected Death Due to Myocarditis, Acta path. et microbiol. scandinav. Supp. 105, p. 30, 1955.

12. Trossman, C. M., and Curtis, C. N.: Fatal Interstitial Cardiopneumonitis in an Infant, U. S. Armed Forces M. J. 7:419, 1956.

13. Dominiczak, K.: Interstitial Nonspecific Myocarditis as Cause of Sudden Death, Pediat. polska 30:571, 1955.

14. van Creveld, S.: Myocarditis in Infants, Nederl. tijdschr. geneesk. 100:63, 1956.

15. Saphir, O., and Field, M.: Complications of Myocarditis in Children, J. Pediat. 45:457, 1954.

16. Rosenbaum, H. D.; Nadas, A. S., and Neuhauser, E. B. D.: Primary Myocardial Disease in Infancy and Childhood, A. M. A. Am. J. Dis. Child. 86:28, 1953.

17. van Creveld, S.; de Groot, J. W. C.; Hartog, H. A. P., and Lie Sing Kiem: Diagnosis and Treatment of Acute Interstitial Myocarditis in Infancy, Ann. paediat. 183:193, 1954.

18. El Gholmy, A.; Aidaros, S.; El Nahawy, M., and Gabr, M.: Fiedler's Myocarditis: Review of Literature with Report of Case, Arch. Pediat. 71:203, 1954.

19. Melbourne Pædiatric Society: Idiopathic Myocarditis in Infancy and Childhood, M. J. Australia 1:76, 1953.

20. Tudor, R. B., and Kling, R. R.: Isolated Myocarditis in Children with 2 Case Reports, Journal-Lancet 72:235, 1952.

21. Luginbuhl, W. H.: Acute Isolated Myocarditis, Case Reports, Children's Memorial Hospital, Chicago, 1955, Vol. 13, p. 3659.

22. Arey, J. B., and Sotos, J.: Unexpected Death in Early Life, J. Pediat. 49:523, 1956.

23. Kibrick, S., and Benirschke, K.: Acute Aseptic Myocarditis and Meningoencephalitis in the Newborn Child Infected with Coxsackie Virus Group B, Type 3, New England J. Med. 255:883, 1956.

24. Bernheim, M.; Gravier, J.; Perrin, A.; Germain, J., and Verney, R.: Two Cases of Idiopathic Myocarditis in the Newborn, Pédiatrie 11: 91, 1956.

25. Gear, J.; Prinsloo, F. R.; Kahn, M., and Kirsch, Z. G.: Myocarditis of the Newborn: Outbreak in a Maternity Home in Southern Rhodesia Associated with Coxsackie Group-B Virus Infection, South African M. J. 29:608, 1955.

26. van Greveld, S., and De Jager, H.: Myocarditis in Newborns Caused by Coxsackie Virus, Ann. paediat. 187:100, 1956.

27. Steeger, A.; Espinoza, J., and Daneri, J.: Chronic Nonspecific Myocarditis of the Newborn, Rev. chilena pediat. 27:49, 1956.

28. Downing, J. S.: Interstitial Myocarditis in Infancy: Report of a Case, J. Iowa M. Soc. 41: 220, 1951.

29. Stoeber, E.: Further Studies on Epidemic Myocarditis of Infants, Ztschr. Kinderh. 71:319 and 592, 1952.

30. Saphir, O.; Wile, S. A., and Reingold, I. M.: Myocarditis in Children, Am. J. Dis. Child. 67:294, 1944.

31. Betke, K., and Harms, I.: The Clinical Syndrome "Encephalomyocarditis, Arch. Kinderh. 146:6, 1953.

32. Verlinde, J. D.; van Tongeren, H. A. E., and Kret, A.: Myocarditis in Newborns Due to Group B Coxsackie Virus: Virus Studies, Ann. paediat. 187:113, 1956.

33. House, R. K.: Diffuse Interstitial Myocarditis in Children, Am. J. Path. 24:1235, 1948.

34. Lind, J., and Hultquist, G. T.: Isolated Myocarditis in Newborn and Young Infants, Am. Heart J. 38:123, 1949.

35. Tedeschi, C. G., and Stevenson, T. D., Jr.: Interstitial Myocarditis in Children, New England J. Med. 244:352, 1951.

36. Saphir, O., and Amromin, G. D.: Myocarditis in Instances of Pneumonia, Ann. Int. Med. 28:963, 1948.

37. Wood, D. A.: Pathologic Aspects of Acute Epidemic Hepatitis, with Especial Reference to Early Stages: Report of a Series of 10 Cases, Including a Case in Which There was Spontaneous Rupture of the Spleen and 6 Cases of Fulminating Disease in Patients Who Had Been Wounded Several Months Previously, Arch. Path. 41:345, 1946.

38. Saphir, O.; Amromin, G. D., and Yokoo, H.: Myocarditis in Viral (Epidemic) Hepatitis, Am. J. M. Sc. 231:168, 1956.

39. Widerman, A.: A Case of Encephalitis and Myocarditis in a Child, J. Albert Einstein M. Center 3:33, 1954.

40. Saphir, O.: Encephalomyocarditis, Circulation 6:843, 1952.

41. Pearce, J. M.: Effect of Hemolytic Toxin of Streptococcus Pyogenes on Viral Myocarditis in Rabbit, A. M. A. Arch. Path. 56:13, 1953.

42. Glaser, R. J.; Thomas, W. A.; Morse, S. I., and Darnell, J. E., Jr.: The Incidence and Pathogenesis of Myocarditis in Rabbits After Group A Streptococcal Pharyngeal Infections, J. Exper. Med. 103:173, 1956.

43. Helwig, F. C., and Schmidt, E. C. H.: A Filter Passing Agent Producing Interstitial Myocarditis in Anthropoid Apes and Small Animals, Science 102:31, 1945.

44. Murphy, G. E.: Evidence that Aschoff Bodies of Rheumatic Myocarditis Develop from Injured Myofibers, J. Exper. Med. 95:319, 1952.

Spontaneous Rupture of the Spleen in Infectious Mononucleosis

Report of a Case

GEORGE F. WAGMAN, M.D., Eloise, Mich.

Although it is well known that the spleen may rupture in infectious mononucleosis either spontaneously or following slight abdominal trauma, only 21 well authenticated cases have been reported in which the spleen was examined histologically. In addition, the literature contains reports of the condition in 20 autopsies in which death resulted from causes other than splenic rupture. It is the purpose of this paper to report a case in which the pathologic findings differ somewhat from those previously described.

Report of Case

The patient, a 25-year-old white male intern, was admitted to this hospital on March 19, 1956, with the chief complaint of sudden diffuse upper and periumbilical pain radiating to the left shoulder and with associated weakness and cold perspiration. He had been well except for mild anorexia beginning about two weeks previously. He gave no history of recent abdominal trauma.

Physical examination revealed enlarged cervical, axillary, and epitrochlear lymph nodes, which were soft but not tender or fixed. The tonsils were enlarged and edematous. Signs of peritoneal irritation were present, and paracentesis yielded blood in the left upper quadrant of the abdomen. Shock developed twice before surgery was performed (five hours after the initial onset of abdominal pain).

At operation the abdominal cavity contained approximately 1000 ml. of blood, and the spleen was four to five times its normal size and had a large posterior subcapsular hematoma and laceration. An accessory spleen measuring 1.5 cm. in greatest dimension lay adjacent to the hilum of the spleen. The spleen, the accessory spleen, and a mesenteric lymph node were removed, and a small wedge of liver tissue was excised for biopsy. By March 26, 1956, the patient was afebrile and had no significant adenopathy. He was discharged five days later.

The majority of the lymphocytes in the peripheral blood on March 19 were atypical and were characteristic of infectious mononucleosis. On June 5 no atypical lympocytes were seen (Table).

The serum bilirubin was 0.4 mg. per 100 ml. on March 19, 0.9 mg. on March 23, and 0.3 mg. on April 18. The remainder of the liver tests, including tests for cephalin-cholesterol flocculation, thymol turbidity and flocculation, alkaline phosphatase, and sulfobromophthalein excretion, were normal. These findings correlated well with the histologic appearance of the liver, which showed no necrosis of parenchymal cells and no evidence of biliary obstruction.

Submitted for publication April 8, 1957.

From the Department of Pathology, Wayne County General Hospital; Resident Physician in Pathology.

Summary of Results of Blood Tests

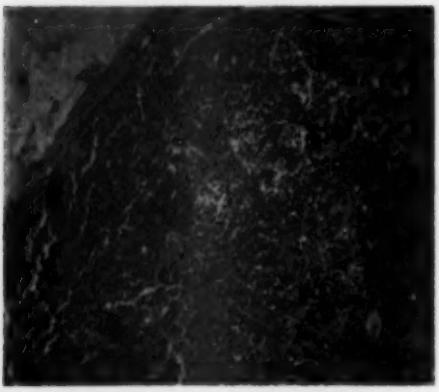
Date, 1956	Hb., Gm.	WBC	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils	Heterophil Test: Presumptive	Heterophil Test: After Absorption with Guinea Pig Kidney
March 19	12.4	7,500	50	45	5			1:448	1:56
22	9.2	10,250	65	32	3				
27	12.7	6,150	56	39	1	2	2		
April 18	13.0	7,200						Less than 1:56	1:14
June 5	12.9	11,850	73	23	3		1		
6								1:14	1:7

Pathologic Findings

The spleen was bisected immediately upon removal, and imprints were made and treated with Wright's and Giemsa's stains. Sections of the spleen, accessory spleen, a mesenteric lymph node, and liver tissue were stained with hematoxylin and eosin, phloxine-methylene blue, Giemsa's stain, a modification of Wilder's silver impregnation method for reticulum, and Prussian blue method for free iron.

Mesenteric Lymph Node.—The architecture was distorted; no definite follicles were visualized, most sinuses were compressed, and sinus tracery was almost obliterated by lymphoid and reticulum-cell hyperplasia of pulp cords. Numerous typical and "atypical" lymphocytes were seen. The proliferation of sinus littoral cells was prominent (Fig. 1). The capsule and the pericapsular adipose tissue were focally invaded by normal and "atypical" lymphocytes. Similar cells were present in the lymph sinuses and the perinodal tissue. Mitotic figures of both lymphoid and reticulum cells were frequent but not atypical. Trabeculae were seen only infrequently and were thin, fragmented, and infiltrated with round cells. The "atypical" lymphocytes that were seen appeared to be identical with the "predominant mononuclear cell" of Smith and Custer [1] and the "specific infectious mononucleosis cell" of Gall and Stout.[2] In sections stained with hematoxylin and eosin, the cells varied from 12μ to 20μ in diameter, were round, and had a moderately faint uniform eosinophilic cytoplasm. In comparison with normal lymphocytes, the cell was larger and had more abundant and fainter-staining cytoplasm. The nucleus was usually central but occasionally slightly eccentric and closely resembled those of normal lymphocytes; an occasional cell had an indented nucleus, and infrequently one had a nucleolus.

Fig. 1.—Section of lymph node. Note distortion of architecture, absence of follicles, compression of sinuses, and hyperplasia of sinus littoral cells. Hematoxylin and eosin; reduced ⅓ from mag. × 120.



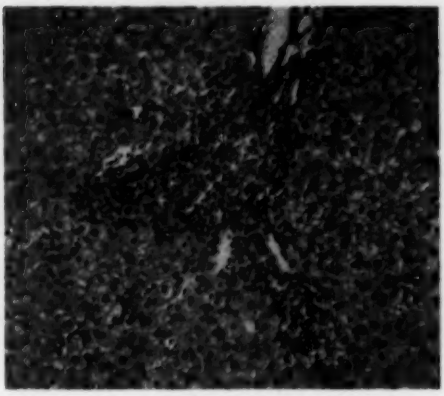


Fig. 2.—Liver. Heavy lymphocytic infiltration in a periportal area. Also note lymphocytes within sinusoids. Hematoxylin and eosin; reduced ⅓ from mag. × 200.

Vascular changes were present similar to those to be described in the spleen.

In general the changes seen correspond closely to those found in the "florid stage" by Gall and Stout and in lymph nodes removed at the height of the disease by Downey and Stasney.[3]

Liver.—There was slight capsular and subcapsular infiltration and moderately heavy periportal infiltration (Fig. 2), with typical and "atypical" lymphocytes and occasional polymorphonuclear leukocytes and eosinophils. Slight cloudy swelling and slight focal fatty infiltration were present, but there was no necrosis of parenchymal cells. There was moderate proliferation of Kupffer cells, and the sinusoids contained many lymphocytes, some of which were

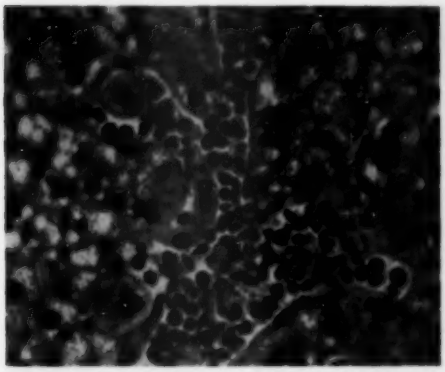


Fig. 3.—Liver. Numerous large atypical lymphocytes in a dilated sinusoid. Also note fatty infiltration of parenchyma. Hematoxylin and eosin; reduced approximately ⅓ from mag. × 540.

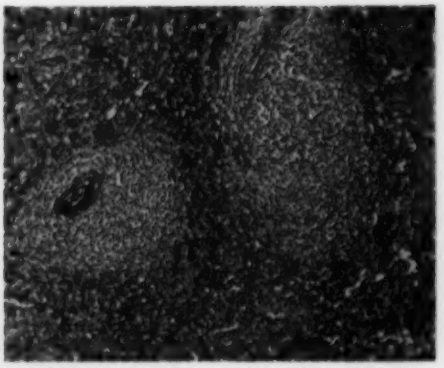


Fig. 4.—Spleen with normal-sized follicles. Note absence of peripheral lymphocytic borders. The follicular borders are sharply defined. Modification of Wilder's reticulum stain; reduced approximately ⅓ from mag. × 120.

atypical (Fig. 3). No evidence of biliary obstruction was seen. These findings correlate closely with those described by Custer and Smith,[4] Allen and Kellner,[5] Ziegler,[6] and Sharp.[7]

Spleen.—The spleen weighed 550 gm. and had a large posterior laceration and a subcapsular hematoma. The capsule was tense; the parenchyma, much softer than usual and moderately congested, and the Malpighian follicles were well visualized.

Microscopically the architecture was maintained, a normal number of follicles being present. The majority of the follicles were of normal size. A few were larger and a few smaller than normal. Practically all follicles were composed entirely of "germinal centers" and were devoid of peripheral lymphocytic collars (Fig. 4). The entire follicle consisted of reticulum cells in various stages of development (Fig. 5). The follicular borders for the most part were fairly well defined but were often irregular and occasionally merged with the adjacent red pulp. Despite an increase in cellularity of the pulp cords by atypical lymphocytes, the sinuses were dilated and the littoral cells, prominent (Fig. 6) and in some sections the sinuses were moderately congested with blood. Typical and atypical

Fig. 5.—Spleen. Central portion of Malpighian follicle, consisting of reticulum cells in various stages of development, a few having mitotic figures. Hematoxylin and eosin; reduced ⅓ from mag. × 480.

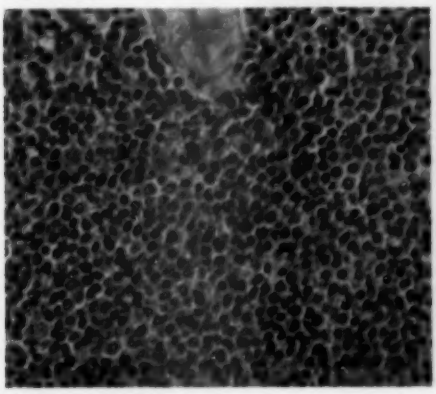
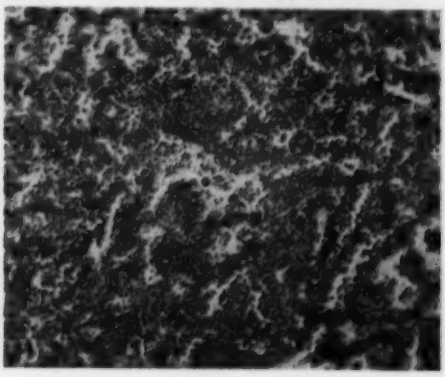


Fig. 6.—Spleen. Note increase in cellularity of the pulp cords, dilatation of sinuses, and prominence of littoral cells. Hematoxylin and eosin; reduced approximately ⅓ from mag. × 120.



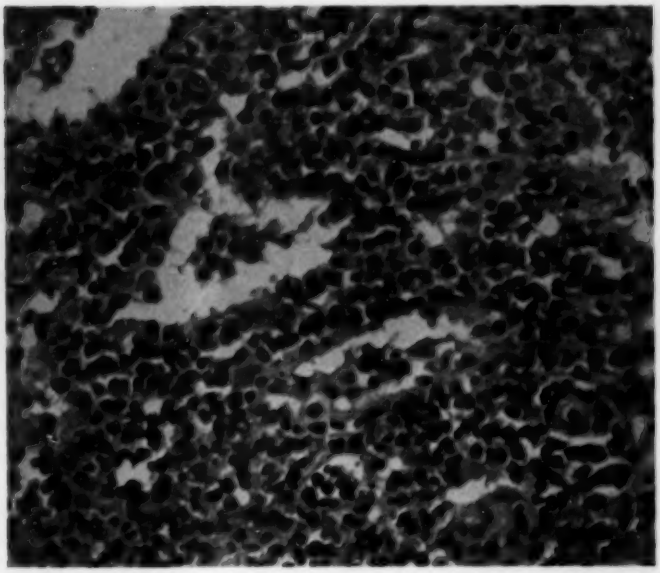


Fig. 7.—Spleen. Large atypical lymphocytes in pulp cords and sinuses. Note increased cellularity of pulp cords. Hematoxylin and eosin; reduced slightly from mag. × 480.

lymphocytes were seen in the sinuses (Fig. 7). Mitotic figures were frequently found in both the follicles and the pulp cords. The capsule and the relatively few remaining fragmented trabeculae were heavily infiltrated with normal and atypical lymphocytes. However, in no area, even at the site of rupture, was the capsule thinned.

Vascular changes described by Smith and Custer, especially the presence of normal and atypical lymphocytes "in the adventitia of small intratrabecular arteries (Fig. 8), and in the subintimal zone of collecting venous sinuses and intratrabecular veins" (Fig. 9), were noted in both the spleen and in the mesenteric lymph node. The acces-

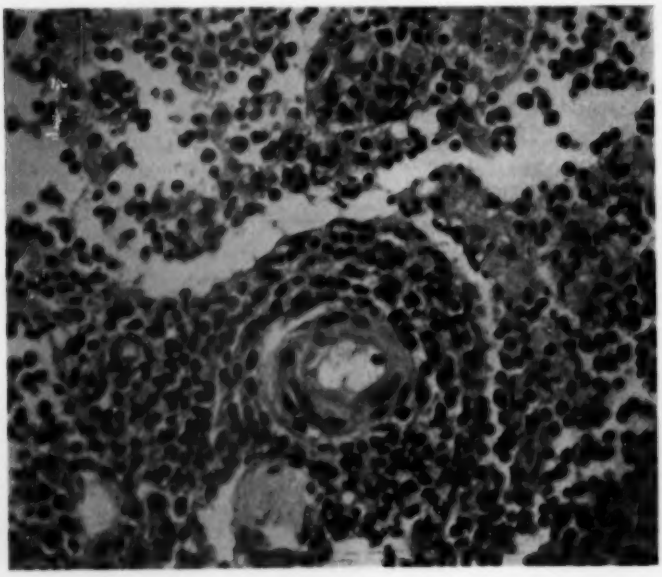


Fig. 8.—Lymph node. Arteriole with adventitial collar of lymphocytes. Note large atypical lymphocytes in adjacent sinuses. Hematoxylin and eosin; reduced slightly from mag. × 240.

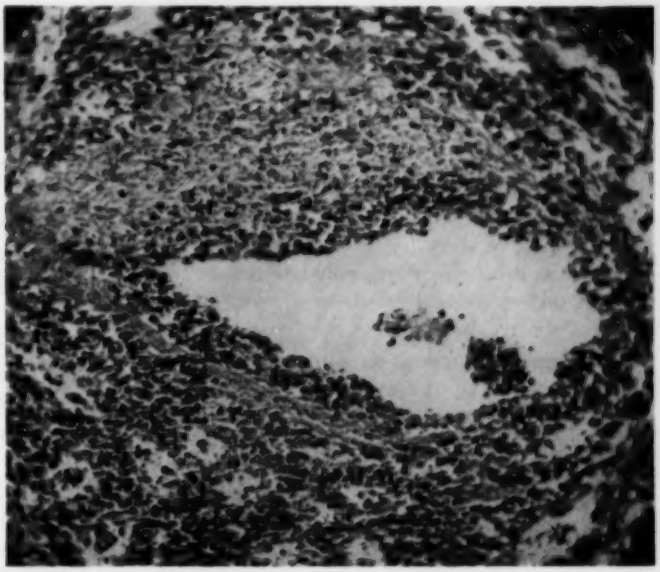


Fig. 9.—Spleen. Lymphocytes present in subintimal zone of an intratrabecular vein. Hematoxylin and eosin; reduced slightly from mag. × 240.

sory spleen showed changes identical with those in the spleen.

Focal collections of vacuolated lipid-containing cells were found at the periphery of an occasional follicle. This was thought to be the result of liquid petrolatum (mineral oil) ingestion and to represent an incidental finding.

Imprints of the spleen showed considerable increase of reticulum cells, with apparent transition between these cells and the numerous atypical lymphocytes characteristic of infectious mononucleosis.

Comment

The histopathologic changes in the spleen described in this case resemble closely those previously reported except for the description of the Malpighian follicles. Smith and Custer found "small, poorly defined follicles, usually without germinal centers, in less than usual numbers per unit area." Darley and associates [8] reported follicles which were "small and lacked germinal centers." In Tudor's [9] case the follicles were small and widely spaced, with practically no germinal centers. Sharp found small widely separated and ill-defined follicles. A "subtotal loss of follicular architecture" was found in Ziegler's case. Similarly, Hicks,[10] Belton,[11] Gulben,[12] and Vaughan and associates [13] reported small inactive Malpighian follicles.

Only occasionally have normal-sized follicles in normal numbers been found. Sullivan and Wasserman [14] reported conspicuous although not particularly enlarged follicles with inactive germinal centers. Kass and Robbins [15] found normal-sized follicles containing atypical lymphocytes in their centers. King [16] reported distinct follicles of the usual size. In one of the two cases of splenic rupture reported by Timmes and co-workers [17] the spleen ruptured following abdominal trauma, less than two weeks after the onset of symptoms. In this case there was a "normal number of Malpighian bodies. In many of them the germinal cells were enlarged and disclosed rather marked reticuloendothelial hyperplasia." Beswick [18] described traumatic rupture of the spleen early in the course of the disease. In her patient the follicles were rather widely separated, and about half of them contained small germinal centers. She felt "that proliferation of cells in the germinal centers is

an early and transient phenomenon, and that necrosis and disintegration of the proliferated cells follow rapidly, so that germinal centers are usually no longer apparent when the spleen is examined later in the disease." In contrast to previous authors who had stressed cellular hyperplasia in the cords of the red pulp, she postulates that "cellular proliferation . . . begins in the marginal zone of the white pulp and later spreads progressively throughout Billroth's cords of the red pulp."

A different hypothesis is suggested by the findings in our patient of Malpighian follicles that were of normal number and size, the entire follicle consisting of reticulum cells, with almost complete absence of mature lymphocytes. It seems more probable in this case that cellular proliferation began and continued in the germinal centers so that eventually a follicle remained, consisting entirely of an active "germinal center." This cannot be assumed to be an early transient phase, since the history, laboratory data, enlarged lymph nodes, and spontaneous rupture of the spleen in the third week indicated that the disease was at its height.

The histopathologic changes in the mesenteric lymph nodes suggest either a stage later in the disease than that in the spleen or that the site of the reticulum-cell hyperplasia may vary markedly in these structures. It seems more probable that the latter view is correct and that in this case the lymph-node hyperplasia is chiefly caused by proliferation of reticuloendothelial cells of sinuses and red pulp, with encroachment upon and loss of lymphoid follicles, while in the spleen there is reticulum-cell hyperplasia of both follicles and red pulp. It is not plausible to assume that the reticulum cells of the germinal centers, normally the chief cells that form lymphocytes, cannot partake in the formation of the atypical lymphocytes seen in infectious mononucleosis. No doubt, if the process had continued, eventually the follicles would have merged with the red pulp, with loss of follicles and replacement by irregular sheets of reticulum cells. Nevertheless, it is seen that the spleen may rupture without trauma at the stage of follicular hyperplasia. From these findings it can be inferred that the histopathology of the spleen and lymph nodes in infectious mononucleosis depends not only on the stage of the disease, but upon the target structure most heavily stimulated, whether it be lymphoid follicles or lining cells of sinuses and pulp reticulum or both of these.

Smith and Custer pointed out that the spleen ruptures during the third and fourth week of the disease, owing to its rapid enlargement and associated infiltration and dissolution of the capsule by atypical lymphocytes. In our patient the capsule was heavily infiltrated with atypical lymphocytes, but at no area did it appear thinner than normal.

Summary

The pathologic findings are described in a patient with infectious mononucleosis whose spleen ruptured spontaneously during the third week of the disease.

The majority of previous reports have emphasized the small inactive Malpighian follicles and marked hyperplasia of the red pulp. In this case the Malpighian follicles were normal in number and size, and practically all were composed entirely of reticulum cells, while hyperplasia of the red pulp was not as conspicuous. A mesenteric lymph node, however, showed different changes, with marked hyperplasia of sinus histiocytes and cord reticulum cells and complete loss of lymphoid follicles.

It is suggested that the histopathology of the lymphoid organs in infectious mononucleosis depends not only on the stage of the disease but upon the target structure most heavily stimulated, whether it be lymphoid follicles or sinus and pulp reticulum, or both.

Department of Pathology, Wayne County General Hospital.

REFERENCES

1. Smith, E. B., and Custer, R. P.: Rupture of the Spleen in Infectious Mononucleosis: A Clinicopathologic Report of 7 Cases, Blood 1:317, 1946.

2. Gall, E. A., and Stout, H. A.: The Histological Lesion in Lymph Nodes in Infectious Mononucleosis, Am. J. Path. 16:433, 1940.

3. Downey, H., and Stasney, J.: The Pathology of the Lymph Nodes in Infectious Mononucleosis, Folia hæmat. 54:417, 1936.

4. Custer, R. P., and Smith, E. B.: The Pathology of Infectious Mononucleosis, Blood 3:830, 1948.

5. Allen, F. H., Jr., and Kellner, A.: Infectious Mononucleosis: An Autopsy Report, Am. J. Path. 23:463, 1947.

6. Ziegler, E. E.: Infectious Mononucleosis: Report of a Fatal Case with Autopsy, Arch. Path. 37:196, 1944.

7. Sharp, M. E.: Infectious Mononucleosis: Report of a Case with Autopsy, J. Path. & Bact. 62:175, 1950.

8. Darley, W.; Black, W. C.; Smith, C., and Good, F. A.: Spontaneous Splenic Rupture in Infectious Mononucleosis: A Case and Pathologic Report, Am. J. M. Sc. 208:381, 1944.

9. Tudor, R. W.: Rupture of Spleen in Infectious Mononucleosis, Brit. M. J. 2:813, 1953.

10. Hicks, S. P.: Rupture of the Spleen in Infectious Mononucleosis: Report of a Case, U. S. Nav. M. Bull. 48:460, 1948.

11. Belton, C. H.: Rupture of the Spleen in Infectious Mononucleosis: Report of a Case, New Zealand M. J. 51:111, 1952.

12. Gulben, S.: Ruptured Spleen in Infectious Mononucleosis, J. Am. M. Women's A. 5:488, 1950.

13. Vaughan, S. L.; Regan, J. S., and Terplan, K.: Infectious Mononucleosis Complicated by Spontaneous Rupture of the Spleen and Central Nervous System Involvement, Blood 1:334, 1946.

14. Sullivan, J. M., and Wasserman, S. E.: Spontaneous Rupture of the Spleen Due to Infectious Mononucleosis, J. A. M. A. 134:144, 1947.

15. Kass, E. H., and Robbins, S. L.: Severe Hepatitis in Infectious Mononucleosis: Report of a Case with Minimal Clinical Manifestations, and Death Due to Rupture of the Spleen, Arch. Path. 50:644, 1950.

16. King, R. B.: Spontaneous Rupture of the Spleen in Infectious Mononucleosis: Report of a Case, New England J. Med. 224:1058, 1941.

17. Timmes, J. J.; Averill, J. H., and Metcalfe, J.: Splenic Rupture in Infectious Mononucleosis, New England J. Med. 239:173, 1948.

18. Beswick, I. P.: The Spleen in Glandular Fever, J. Path. & Bact. 70:407, 1955.

Correction

In the article "Histochemical Studies of Some Keratotic and Proliferating Skin Lesions: 1. Metachromasia," by Dr. Herbert Fanger and Miss Barbara E. Barker, in the August issue of the ARCHIVES, the photomicrographs for Figures 3 and 4 should be in reverse order—the photomicrograph in Figure 3 should be over the legend for Figure 4, and the one for Figure 4, under the legend for Figure 3.

The Intrahepatic Distribution of Copper in Relation to the Pathogenesis of Hepatolenticular Degeneration

L. LAHUT UZMAN, M.D., Boston

The deposition of excessive amounts of copper in various body organs constitutes a prominent feature of the disturbance in copper metabolism in hepatolenticular degeneration.[1-5] Yet, the temporal relationship between the deposition of copper and the other biochemical abnormalities (aminoaciduria, specific peptiduria) on one hand, and the development of clinical manifestations of the disease on the other, has received scant attention. Studies pertinent to the copper abnormalities have confined themselves to the delayed disappearance of albumin-bound copper from serum,[6] the deficiency of caeruloplasmin,[4,6,7] and the failure of orally administered Cu^{64} to appear in the caeruloplasmin fraction.[6] It is also recognized that orally ingested copper has to go through a tissue stage of unknown nature and duration[7,8] before it appears in the urine as an oligopeptide or peptide-conjugated copper chelate.[9,10]

It is evident, however, that the interpretation of results, based on the changes in the amount and state of binding of copper in serum and urine, is largely dependent on a knowledge of preexisting copper stores in the patient's body, especially the liver, at the time of such study. The necessity of information in this respect becomes more urgent in view of our previous report on the high affinity for copper of specific liver proteins in Wilson's disease.[11] As was pointed out in that study, the rate of uptake of albumin-bound copper from the serum by the liver and the release of tissue-bound copper will depend on the residual avidity of the liver proteins for additional copper binding. Thus, different conditions would be expected to prevail, depending on whether the tissues (predominantly liver, in short-term experiments) were already saturated with respect to their binding capacities or not.

Furthermore, the stage of the disease at which copper deposition in the tissue occurs appears to be of paramount importance in any consideration of the pathogenesis of hepatolenticular degeneration. Although nobody seriously ascribes any more the development of cirrhosis in the liver or the development of nervous signs to "intoxication" by copper per se, the relationship of the clinical involvement to the degree of copper deposition has remained an unanswered question. It is a question of whether the dramatic improvement of neurological signs upon initiation of dimercaprol (BAL) therapy can be ascribed solely to the amount of offending copper removed from the body, inasmuch as the amounts of metal thus eliminated constitute an infinitesimal fraction of the patient's estimated total body stores of copper.[4,12] Furthermore, there has been difficulty in maintaining improvement in some cases with continued dimercaprol therapy. Finally, the inability to reverse or halt the progress of the liver cirrhosis by dimercaprol treatment in all cases so far studied points to a different pathogenesis than the simple overaccumulation of copper in these patients, possibly because other factors are involved. If the copper deposition in tissues were due to a peculiarity in

Submitted for publication March 11, 1957.

From the Department of Neurology, Harvard Medical School, and the Neurological Unit, Boston City Hospital.

Supported by a grant (B-712) from the National Institute of Neurological Diseases and Blindness, U. S. Public Health Service.

the physical-chemical state of copper in the plasma after large amounts of the metal had been absorbed by the intestinal tract,[4,8,12] as suggested by some, then a uniform deposition in all cells in an organ like the liver would be expected. If, on the other hand, the copper content of the liver depended on the presence of some intracellular protein constituent that was produced at some stage of cellular development in the process of lobular regeneration, inequalities in the copper content of different lobules would be expected. The latter appeared probable, in view of the specific binding properties residing in liver proteins in this disease.[11]

The present investigation was undertaken to ascertain if such inequalities did indeed exist in the liver lobules of patients with hepatolenticular degeneration, and an attempt was made to determine whether the regenerating liver lobule also possessed the protein fraction responsible for excessive copper binding.[11] The latter consideration assumed special importance because the presence of the copper-binding fraction would not only indicate the transference of the copper-binding stigma to regenerating cells but would label these cells also as potentially new and avid reservoirs for further deposition of additional copper. It was also felt that another question of vital importance in the formulation of a pathogenesis for the disease might thus be answered, i. e., the question of how early in the disease does excessive copper deposition in the liver occur. Histochemical studies aimed at the detection of copper in the organs of cases of hepatolenticular degeneration at postmortem examination are already on record.[13,14] These, however, are not germane to the questions raised here, as they utilize methods not designed to yield intracellular localization and fail to provide any assurance that contamination with extraneous copper during the processing of the tissues was avoided. These studies also present the end-stages of hepatolenticular degeneration and do not contribute to defining the relationship of the amount and pattern of copper deposited to the course or phase of the clinical picture.

Our own studies deal with liver tissue obtained from two patients at autopsy and six patients by biopsy and show unequivocally that there is a very marked difference in the copper content of various liver lobules in individual patients and that this deposition follows characteristic patterns. Furthermore, the deposition of copper in the liver may already be present in asymptomatic patients in whom the characteristic aminoaciduria of the disease could be demonstrated as an additional metabolic stigma. In addition, copper-free regenerating lobules appear to possess the affinity to bind copper by virtue of their demonstrated content of the copper-binding protein constituent (Fraction X).[11]

Report of Cases *

CASE 1.—A 43-year-old former truck driver in whom the disease had started with the picture of pseudosclerosis 13 years ago, without evidence of liver disease. (A brother who had also been under observation died with the disease three years previously.) The case history of this patient was extensively reported previously.[15,16] For the past five years the patient had been treated with weekly injections of dimercaprol.[16] During the last year of his malady he developed increasing signs and symptoms of portal hypertension and liver failure, in spite of the fact that the tremor which had been quite severe 10 years ago was only minimally present even a few days before death. The patient died in hepatic coma.

The liver was removed four hours postmortem, and portions were immediately frozen. The liver was hard, nodular, and grossly atrophic. Histologically the picture was one of severe Laennec type of cirrhosis. Marked variation in the copper content of individual lobules was demonstrated (Figs. 1 and 2).

CASE 2.†—An 18-year-old woman in whom the disease had its onset at 7 years of age, with progressive clumsiness of upper and lower extremities,

* The pertinent aspects are summarized in the Table.

† Dr. H. Yannet, Medical Director, Southbury Training School, Southbury, Conn., made specimens from the postmortem examination available in the frozen state.

Summary of Pertinent Features of Cases

Case	Age	Duration of Disease	Type of Neurological Involvement	Family History	Amino-Aciduria and Peptiduria	Liver at Postmortem or Biopsy		K-F Rings
							Copper	
1	43	13 yr.	Pseudosclerosis, liver biopsy negative 10 yr. ago[14]	One older brother died with identical picture[13, 14]	Severe amino-aciduria and cupruria[13, 14]	Severe cirrhosis	++++	+
2	18	11 yr.	Dystonia with rigidity, choreiform movements, dysarthria, mental deterioration	One sibling, healthy	Not studied	Severe cirrhosis with fatty degeneration	++++	+
3	14	1 yr.	Dystonic rigidity, severe	One sibling age 12, severe cirrhosis and K-F rings; one sibling age 6, K-F rings but asymptomatic; one sibling age 3, hepatosplenomegaly	Amino-aciduria more than 1.0 gm. α amino N per 24 hr.; specific peptiduria	Severe cirrhosis	++++	+
4	14	3 mo.	Dystonic rigidity, dysarthria, tremor	Cases 4-8 are siblings	687, 806, 1,127 mg. α-amino N per 24 hr.; peptiduria +	No cirrhosis	++	+
5	15	? Asymptomatic	Early dystonia	3 cousins, all children of paternal uncle, died with juvenile hepatolenticular degeneration	665 mg. α-amino N per 24 hr.; peptiduria +	No cirrhosis	+	+
6	12	Asymptomatic	None		259 mg. α-amino N per 24 hr.; peptiduria +	No cirrhosis	−	−
7	11	? Asymptomatic	Early dystonia		296 mg. α-amino N per 24 hr.; peptiduria +	No cirrhosis	+	+
8	4	? Asymptomatic	None		185 mg. α-amino N per 24 hr.; peptiduria +	Fatty degeneration with early cirrhosis	−	−

Fig. 1.—Blocks of fresh liver tissue from Case 1, immediately dropped into the rubeanic acid reagent-fixative. There is a marked difference in the copper contents of individual lobules. Those lobules staining black represent heavily copper-laden lobules; lobules without copper are seen as a much paler gray (pink-orange in reality), while the fibrous bands are white; reduced approximately ½ from mag. × 6.

"nervousness," dysarthria, drooling at the mouth, and choreiform movements. At 12 years of age the patient already exhibited dystonic extensor rigidity of legs, with plantar flexion and inversion of feet and with extensor plantar reflexes. Bilateral Kayser-Fleischer rings were noted. There was marked euphoria and mental deterioration. The patient did not tolerate therapy with dimercaprol or edathamil calcium-disodium (Calcium Disodium Versenate). Her course was one of progressively increasing dysarthria, a coarse action tremor, drooling, and rigidity. Three years prior to death she began to show laboratory evidence of mild hepatic involvement (icteric index, 7.0; cephalin flocculation, 3+; albumin, 3.4%; globulin, 2.8%). There were no clinical signs of liver disease. Terminally the patient became quite incapacitated and bedridden and died of pneumonia in a state institution.

Necropsy 12 hours after death showed severe portal cirrhosis with splenomegaly, but without ascites. There was marked difference in the copper content of different lobules as demonstrated histochemically (Figs. 3 and 4).

Case 3.—A 14-year-old girl who was admitted with a one-year history of behavior changes, progressive clumsiness of the hands and legs, and assumption of "catatonic" postures. On examination she was found to be in severe dystonic rigidity, with dystonic postures of all four extremities. The rigidity involved all facial musculature, as well as tongue and pharyngeal muscles. Severe dysarthria and the characteristic expressionless facies of this disease were noted. Bilateral extensor plantar reflexes were easily elicited. She had bilateral Kayser-Fleischer rings. A hard nodular liver edge was felt 4 cm. below the costal margin. A daily amino acid output in excess of 1 gm. of α-amino nitrogen was noted on three occasions, while a moderate generalized amino-aciduria was present throughout her hospitalization (400-600 mg. α-amino nitrogen/24 hours). Chromatography confirmed the generalized nature of the amino-aciduria and demonstrated the presence of specific peptiduria.[9,10]

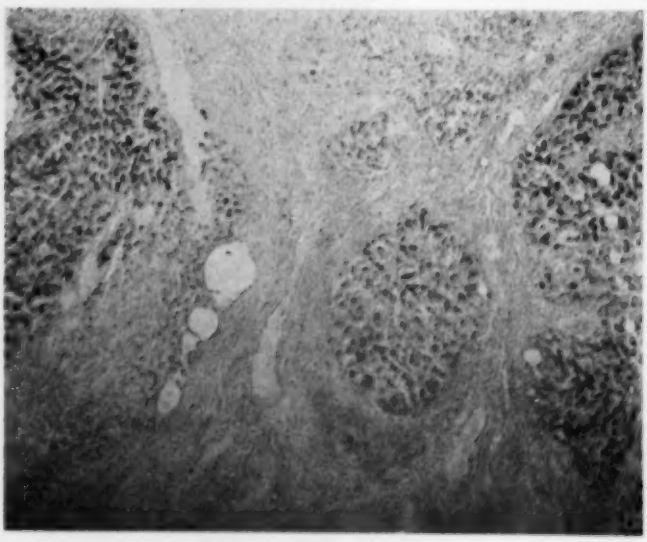


Fig. 2.—Rubeanic-acid-stained section of liver from Case 1, showing lobules with markedly different copper content. No counterstain; reduced approximately 15% from mag. × 180.

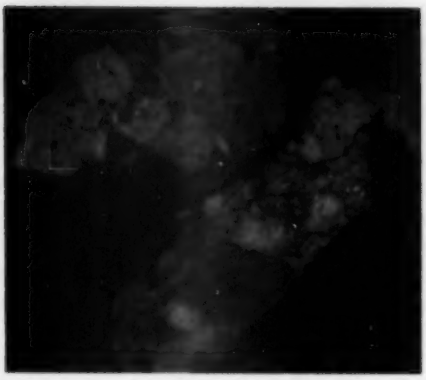


Fig. 3.—Blocks of fresh liver tissue from Case 2 immediately dropped into the rubeanic acid reagent-fixative. Again the marked inequality in the copper content of liver lobules is quite apparent; reduced approximately 40% from mag. × 6.

Two younger siblings (12 and 5 years old) who were also examined showed Kayser-Fleischer rings. The older one had a liver edge palpable below the umbilicus, with clinical and laboratory evidence of severe hepatic cirrhosis and esophageal varices. The younger was asymptomatic. Neither of the siblings had any neurological signs or symptoms. Both, however, exhibited the gross generalized amino-aciduria characteristic of the disease (average of 480 and 270 mg. α-amino nitrogen on three specimens).

The patient did remarkably well under dimercaprol therapy and alternating high- and low-protein diet. The rigidity decreased, so that she could walk and talk. This improvement was only maintained, however, for about eight months, after which time dysarthria and severe rigidity of all extremities became again quite pronounced in spite of continued dimercaprol therapy. The patient began to have frequent generalized seizures, poorly controlled by anticonvulsant medication.

A liver biopsy was performed during her initial hospitalization, while improvement was apparent and after the second injection of dimercaprol. The liver specimen showed evidence of severe cirrhosis, with massive copper deposition in parenchymal cells (Figs. 5 and 6).

Case 4.—A 14-year-old boy was admitted with a three-month history of progressive tremulousness in the hands and slurring of speech. Past history was noncontributory. The patient was one of five siblings, aged 15, 12, 11, and 4, respectively, and all were allegedly in good health. However, the diagnosis of Wilson's disease was entertained even before the present admission because three cousins had died with hepatolenticular degeneration (the patient's father and the cousins' father were brothers). On examination the patient demonstrated bilateral Kayser-Fleischer rings, greenish-brown in color and 2 mm. in width at their widest point. The liver edge was hard and palpable 4 cm. below the right costal margin. There was the characteristic fixed facial expression, with retraction of the upper lip. Speech was slow and slurred. A rapid irregular tremor of the hands and fingers was observed with arms extended. There was a plastic rigidity in all four extremities, more marked

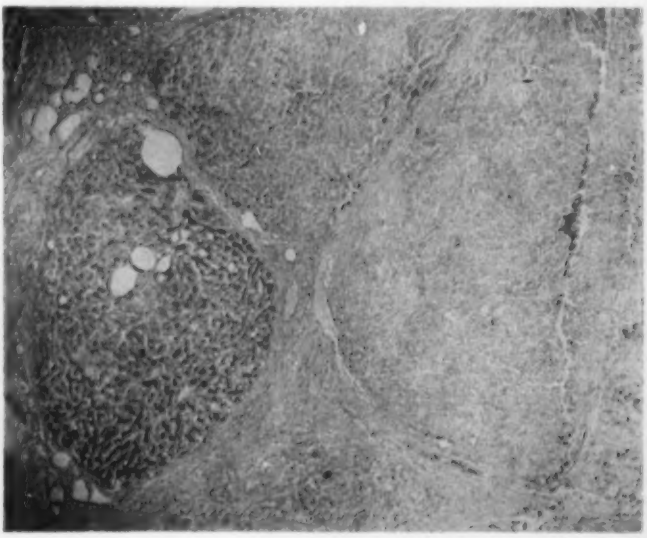


Fig. 4.—High power of appearance of liver in Case 2. The difference in the copper contents, as judged by the black intracellular deposits, between individual lobules is not as pronounced as in Case 1. No counterstain; reduced approximately 15% from mag. × 180.

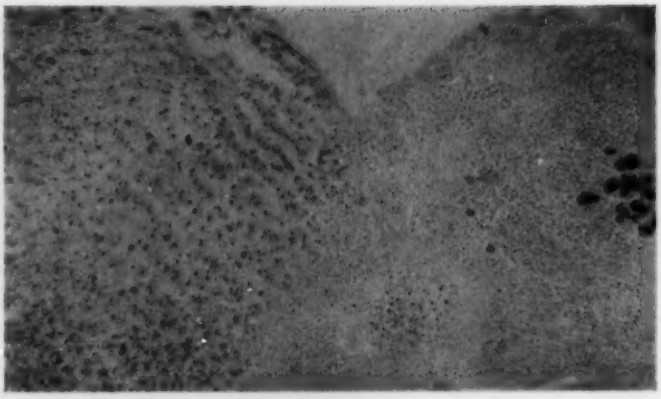


Fig. 5.—Liver biopsy specimen from Case 3, directly fixed-stained with the rubeanic acid reagent. Island of copper-laden liver cells trapped in a mass of fibrous tissue contrasts with lobule on the left, showing moderate copper deposition. No counterstain; reduced approximately 15% from mag. × 200.

in the arms. The right foot was held in an inverted position. The gait was stiff, without associated movements of the arms. Motor power was good throughout. Reflexes were equal and brisk throughout. The left plantar reflex was extensor. The urine was negative for sugar, ketones, and albumin. Sediment showed tyrosine crystals. Urinary α-amino nitrogen values on three successive days were 687 mg., 896 mg., and 1127 mg. per 24 hours. Liver function studies showed cephalin flocculation, 4+; formol gel, +; icteric index, 6.0; prothrombin time 86%; total protein, 7.8 gm. per 100 cc., with an albumin: globulin ratio of 3.1. Lumbar puncture disclosed a clear colorless fluid under an initial pressure of 110 mm. water. There were 2 lymphocytes; the Pandy test was negative; total protein was 25 mg. per 100 cc., and the Hinton was negative. The colloidal gold sol test was recorded as 0122211000.

A liver biopsy was performed with the usual transpleural-transphrenic approach. Histological examination revealed early cirrhosis, with minimal fatty metamorphosis. Histochemical treatment of the biopsy specimen disclosed large amounts of intracellular copper deposits within some lobules, whereas others were spared (Figs. 7 and 8). Those lobules which were spared exhibited marked inequalities in the nuclei of liver cells, some nuclei appearing very pale and swollen.

Case 5.—A 15-year-old girl, older sibling of Case 4. She had been considered quite normal by

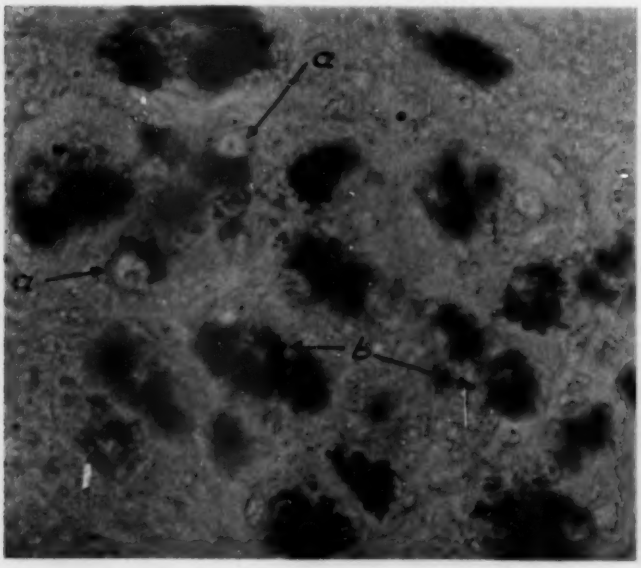


Fig. 6. — High-power view of copper-containing liver cells from Figure 5. The characteristic distribution of copper within cells is well illustrated in this cluster that contains examples of different degrees of copper accumulation. (*a*) Perinuclear "caps." (*b*) Perinuclear plaques, while the other cells show diffuse copper deposition. No counterstain; reduced approximately 15% from mag. × 1200.

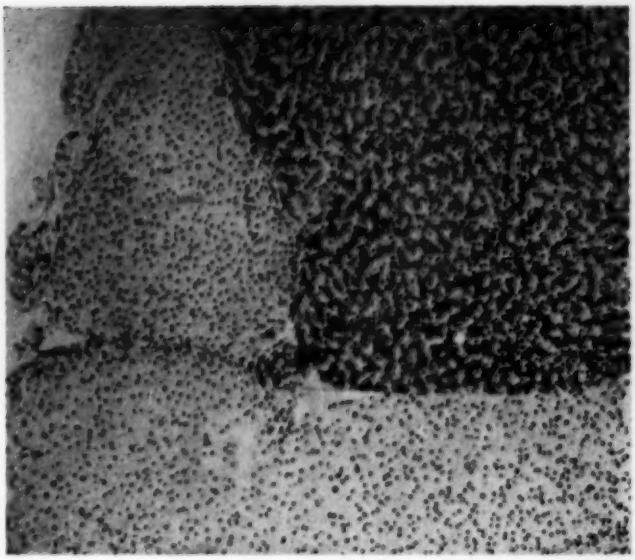


Fig. 7.—Liver biopsy specimen from Case 4, directly fixed-stained with the rubeanic acid reagent. There is no evidence of cirrhosis, but the marked irregularity in black copper-rubeanate deposits between lobules is quite striking. The irregularities in the liver-cell nuclei in the lobules without copper deposits should also be noted. Counterstained with 0.3% aqueous cresyl violet; reduced approximately 15% from mag. × 240.

her family, and her study was only initiated on discovery of the disease in Case 4. The patient was quite intelligent but tended to display euphoria. She exhibited greenish-brown Kayser-Fleischer rings about 2.0 mm. in width bilaterally. The liver was palpable 2 cm. below the right costal margin, with a smooth hard edge. Neurological examination indicated the presence of some slurring of speech, a fixed grin with retraction of the upper lip and maintenance of the mouth in a half-opened position. Hyperpronation of the left forearm when the arms were extended forward and some clumsiness in performing alternating movements were noted. There was an extensor plantar response on the left side. No other neurological abnormalities were noted. Urinary amino acid output was quite elevated (665 mg. α-amino nitrogen/24 hours). The urine (specific gravity, 1026) was free of glucose, acetone, and albumin, with an unremarkable sediment. Liver function

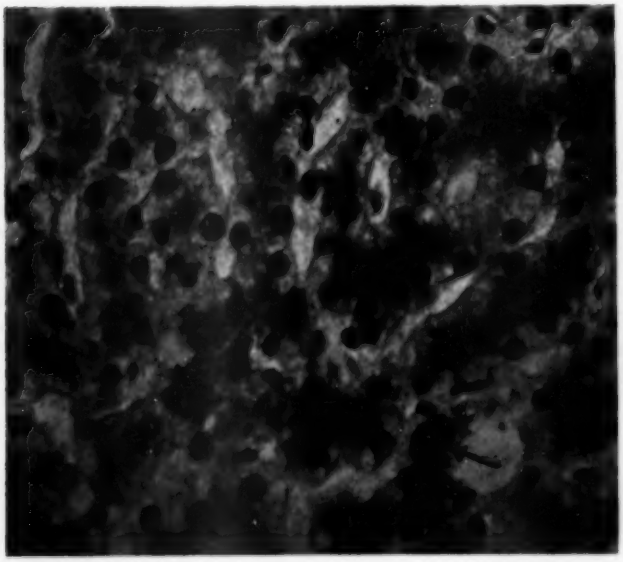


Fig. 8. — High-power view of copper-laden lobule in Figure 7. The deposition of copper-rubeanate appears to be localized to the side of the liver cytoplasm adjacent to the bile ducts. The liver cells show all stages of copper deposition. The arrow indicates the characteristic perinuclear plaques. Counterstained with 0.3% aqueous cresyl violet; reduced approximately 15% from mag. × 800.

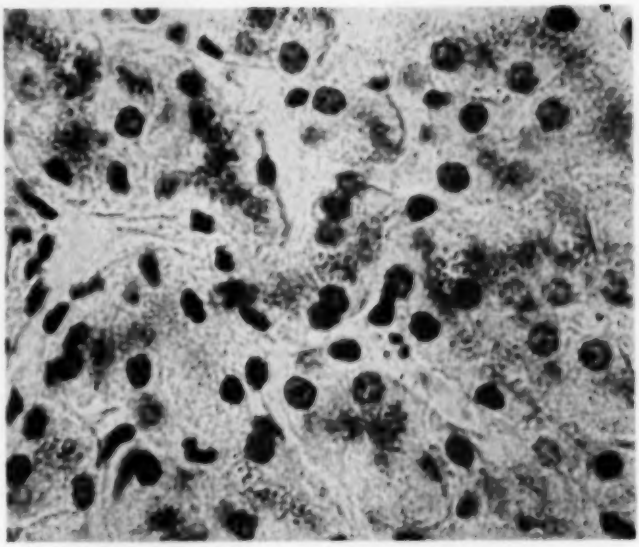


Fig. 9.—Liver biopsy specimen from Case 5, directly fixed-stained with rubeanic acid reagent, showing the fine deposits of copper rubeanate in liver cells, while coarser granules of deposit outline the biliary canals. Counterstained with 0.1% ethanolic cresyl violet; reduced approximately 15% from mag. × 1200.

studies showed no significant abnormality, with a cephalin flocculation, +; prothrombin time, 16.0/14.0 seconds.

A liver biopsy failed to show evidence of cirrhosis but did indicate increased amounts of copper deposition in liver cells (Fig. 9), though much less than in Case 4.

Case 6.—A 12-year-old boy, sibling of Cases 4 and 5, was admitted for study because of the family history. He had been entirely healthy to the present time and was considered quite normal by the family. The neurological examination was entirely negative. Examination failed to reveal Kayser-Fleischer rings. There was no clinical or laboratory evidence of liver disease. The urine showed no sugar or albumin. The boy had to be considered normal, except for an elevated amino acid excretion in the urine (259 mg. α-amino nitrogen /24 hours) and the presence of questionably increased copper in his liver, as evidenced by the histochemical re-

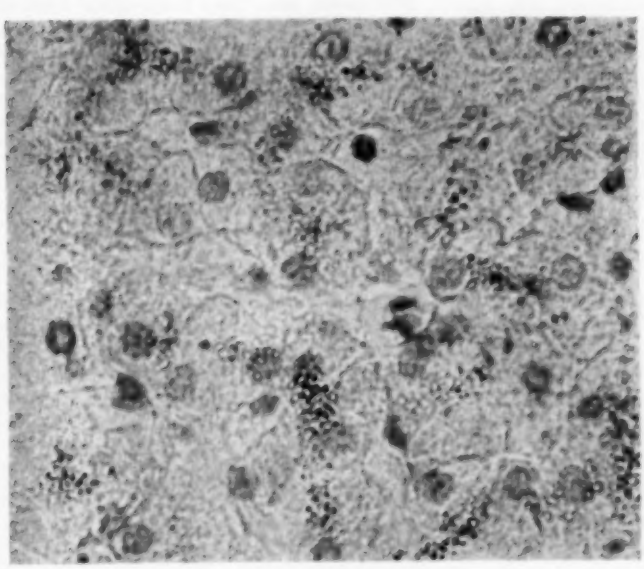


Fig. 10.—Liver biopsy specimen from Case 7, showing increased copper content of liver cells. The deposit is much finer than in the other cases. Counterstained with 0.1% ethanolic cresyl violet; reduced approximately 15% from mag. × 1200.

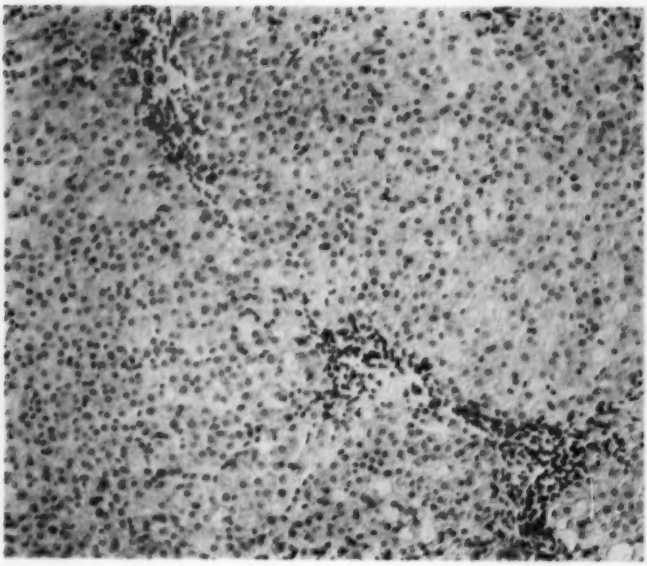


Fig. 11.—Liver biopsy specimen from Case 8, directly fixed-stained with rubeanic acid and counterstained with 0.5% aqueous cresyl violet. The copper deposition is hardly detectable and presumably not abnormally increased. Yet the heavy fibrous cordons, the fatty degeneration, round-cell infiltrates, and cellular unrest present a picture not unlike posthepatitic liver damage with early cirrhosis; reduced approximately 15% from mag. × 200.

action for copper carried out on a liver biopsy specimen.

The liver specimen showed no evidence of cirrhosis or fatty degeneration.

Case 7.—An 11-year-old boy, sibling of the above, who had been considered entirely normal by his family and had done well in school. The boy was intelligent and cooperative. Examination disclosed golden-yellow Kayser-Fleischer rings bilaterally, especially wide (2 mm.), along the superior border of the cornea. The liver edge was just palpable, with a smooth nontender edge at the right costal margin. Neurological examination was negative, except for an extensor plantar response on the left. Prothrombin time was 13.9/12.9 seconds; cephalin flocculation, 0. Urine was free of sugar, acetone, and albumin. Urinary α-amino nitrogen excretion was 296 mg/24 hours.

A liver biopsy specimen failed to show cirrhosis but did show accumulation of copper (Fig. 10).

Case 8.—A 4-year-old girl, youngest sibling of the above four children. The patient's growth and development had been normal. The physical and neurological examinations were entirely negative. There was no clinical or laboratory evidence of liver disease. Urine was free of sugar, acetone, and albumin. Urinary excretion of α-amino nitrogen was 185 mg/24 hours (control levels for this age range between 50-150 mg/24 hours).

Liver biopsy was performed after administration of cyclopropane-ether anesthesia. The specimen obtained showed evidence of increased periportal connective tissue and bands of interlobular fibrous tissue compatible with early cirrhosis. There was also some fatty degeneration evident, while the copper deposition in the liver was borderline normal (Fig. 11).

Material

Liver tissue from Cases 1 and 2 was available in the fresh unfixed state at autopsy. The tissue was frozen in solid carbon dioxide (Dry Ice) until histochemical and electrophoretic studies could be carried out. Liver biopsy specimens obtained in Cases 3-8 were treated with the rubeanic acid reagent immediately on removal from the Vim-Silverman liver biopsy needle.‡ The tissue recovered at biopsy was insufficient for electrophoretic studies.

Methods

Histochemical localization of copper was effected on thinly sliced (2×2 mm.) blocks of fresh tissue removed at autopsy and on fresh liver biopsy specimens. The sections were immediately dropped into 0.1% rubeanic acid in 70% alcohol (ethanol) and processed as previously described.[8] Under these conditions no contamination with extraneous copper was possible. Some of the sections

‡ Dr. Stanley Wolfe, Thorndike Laboratories of the Boston City Hospital, cooperated in performing these liver biopsies.

were counterstained with an 0.1% ethanolic solution of cresyl violet, others, with an 0.3% aqueous solution of the same dye. Cedar oil was avoided throughout the procedure, as this proved to extract some of the copper rubeanate from the sections. Xylene was found to serve admirably both in paraffin embedding and in the clearing of mounted sections.

Dissection of Individual Liver Lobules and Electrophoretic Study.—Since our avowed purpose was to compare those liver lobules that contained large amounts of histochemically detectable copper with those that did not, in order to ascertain differences in the electrophoretic patterns of soluble liver proteins, individual lobules were separated and processed in the following manner. Rectangular blocks of liver tissue 20 mm. long, 10 mm. wide, and 6-8 mm. deep were cut off the frozen organ sample. These were placed in chilled chemically clean Petri dishes and cut carefully to yield two halves (20×10×4 mm.), taking care that each half presented a perfectly smooth surface at the plane of division. One of the blocks was dropped immediately into the rubeanic acid reagent, while the other half was returned to a −20 C freezing chest. After the staining had progressed through the alcohol rinse stage,[5] the stained block was laid next to the frozen unstained sample. Those lobules which had been divided in two by the plane of sectioning were easily identified. Using the stained block as guide, the unstained half of each of the copper-containing and copper-free lobules was scooped out with a stainless steel microspatula and immediately transferred to an ice-chilled Pyrex test tube. Pooled liver lobules representing copper-containing and copper-free lobules (four to five of each, representing a total of 40-60 mg. fresh weight) were homogenized with powdered Pyrex glass and 0.3 ml. of NaCl-Na acetate buffer (pH 7.1, 0.1 M) and centrifuged at 2000 g to obtain the soluble extracts. The supernatants were subjected to paper electrophoresis in the Spinco-Durrum type apparatus, using 0.05 M ammonium acetate (pH 7.5) buffer. Runs were effected, as previously described,[11] at 500 volts for 80 minutes. At the end of this period the buffer pH was 6.9.§ Strips were thoroughly oven-dried at 120 C for 30 minutes to volatilize the ammonium acetate and then sprayed with 0.2% ninhydrin in butanol. The color was developed by heating in an oven for 10 minutes at 110 C. Some strips were also stained for copper, using 0.5 aqueous diethyldithiocarbamate as the spraying reagent. The electrophorograms were scanned with the Spinco Analytrol automatic recording-scanning photometer, using a 580 mμ filter.

§ Presumably due to loss of ammonia as a result of heat generated by the current during the process. Ammonium ions could be detected (Nessler's reagent) in water droplets condensing on the inner surface of the cover of the Spinco-Durrum cell.

Results

Histochemical Localization of Copper.—In the liver tissue removed at autopsy in Cases 1 and 2, as well as the biopsy specimens from Cases 3, 4, and 5, the distribution of copper was remarkably similar. The inequality in the copper deposition was even evident on gross examination (Figs. 1 and 3). Older lobules stained deeply green-black to black, while regenerating lobules discretely outlined by pale fibrous bands were white or yellowish-white. Some lobules showed an intermediate stage, appearing a pale green-gray. On microscopic examination, the amount of deposited copper in each lobule usually appeared uniform with respect to a given lobule. But there were wide differences in the apparent copper contents of adjacent lobules (Figs. 2, 5, and 7). In some lobules whose copper content appeared intermediate, maximal copper deposits were seen in the liver cells at the periphery of lobules. It was possible to recognize that those lobules which had maximal copper by histochemical criteria also appeared to be the oldest or those undergoing destruction (some fatty degeneration, necrosis), while those that appeared relatively free of copper showed the histological criteria of regenerated liver lobules. Occasionally, as in Case 3, small islands of liver cells trapped between heavy cordons of fibrous tissue showed maximal copper deposition (Fig. 5). In none of our material was copper ever detected in a Kupffer cell, even in lobules that appeared to be undergoing necrosis.

On detailed examination of the cytochemical characteristics of the copper distribution within cells, the following observations were made. The intracellular distribution of copper density appears to have distinct characteristics:

(*a*) In lobules that showed increased amounts of histochemically detectable copper there was a very fine deposition of tiny granules of black precipitate throughout the liver-cell cytoplasm. These gave a peculiar

green-gray color effect in sections without counterstain and were best observed in thicker sections (15μ-30μ) (Figs. 2, 4, 9, and 10).

(*b*) The commonest appearance, even in the lobules that contained minimal amounts of copper, was the accumulation of the black precipitate in the form of perinuclear plaques. These usually formed a "cap" around one pole of the nucleus (Fig. 6), then extended and coalesced to form almost an uninterrupted perinuclear layer, obscuring the nucleus from view (Figs. 6 and 8). Thus, in sections without any counterstain one had the impression at low-power examination that the nuclei appeared to be stained black, while with cresyl violet counterstaining intranuclear detail could be made out without difficulty at various levels of focus.

(*c*) In the most heavily involved lobules the copper density extended from the perinuclear area with decreasing intensity toward the periphery of the cell, the black granules tending to become less coarse as the periphery of the cell was approached. In two early cases (Cases 4 and 5) the copper deposits were arranged in that segment of liver-cell cytoplasm closest to the bile ducts (Figs. 8 and 9). It appears worthy of note that in the biopsy specimens from very early examples of the disease (Cases 4, 5, 7, and 8) marked inequality in the size of liver-cell nuclei, even in lobules not containing any copper, was a regular feature. There were many nuclei that were very poorly stained with hematoxylin or cresyl violet; others exhibited, in addition, vacuoles within the nuclei where chromatin was very poorly defined.

Electrophoretic Patterns of Liver-Soluble Proteins.—Liver lobules were processed as described in the previous section. In Case 1, where there was a very marked difference in the copper content of various lobules (Fig. 1), the soluble proteins of groups of lobules with increased copper content was compared with groups that had histochemically no detectable copper. It became apparent that those lobules that had large amounts of copper showed a high Fraction X content, while those which had no detectable copper had a much reduced Fraction X peak, although this fraction was still present (Fig. 12). The presence of this fraction appears to distinguish the soluble liver proteins of the patient with hepatolenticular degeneration from the cirrhotic and the normal.[11]

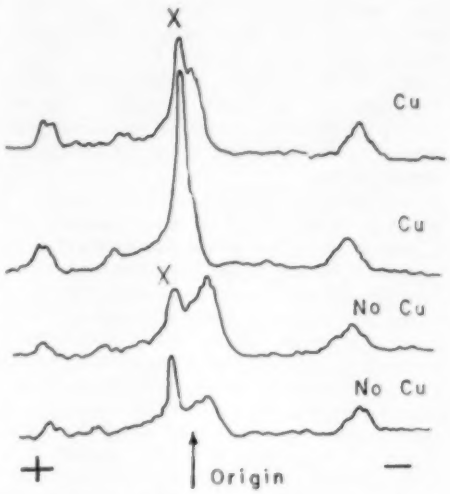


Fig. 12.—Electrophorograms scanned with the Spinco-Analytrol, showing the differences in the soluble liver proteins from copper-rich and copper-poor lobules in Case 1. Ninhydrin stained. The two upper patterns from copper-rich lobules have a pronounced elevation in the Fraction X, while the two lower electrophorograms, representing copper-poor lobules, show a decreased but persistent Fraction X.

In Case 2, where the contrast in copper content of various lobules was not as marked as in Case 1 (Fig. 3), various liver lobules were grouped according to histochemically apparent copper content (ranging from 4+ copper to 1+ copper). The lobules were processed for their respective soluble proteins and electrophoresed under identical conditions as in Case 1. It became apparent that the content of the Fraction X of various lobules was proportional to the amount of histochemically detectable copper content, those lobules with the highest copper content also having the highest proportion of Fraction X (Fig. 13). Again, even in that group

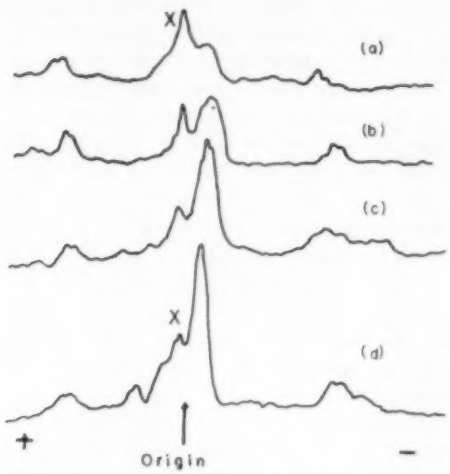


Fig. 13.—Electrophorograms of soluble liver proteins from liver lobules in Case 2. Ninhydrin stained. Pattern (*a*) represents lobules with 4+ copper, (*b*) lobules with 3+ copper, (*c*) lobules with 2+ copper, and (*d*) lobules with + copper deposits detectable histochemically. If the peak immediately beyond the point of origin toward the cathode is used as an internal standard to compare the amount of Fraction X in various lobules, it becomes immediately apparent that the amount of Fraction X is proportionately highest in lobules with maximal copper deposition and least in those that are poorest in copper content.

of lobules containing only minimal amounts of copper, the X-peak was nevertheless present.

In the electrophorograms of Case 1 and Case 2, copper was only present in detectable quantities by the diethyldithiocarbamate reagent in the electrophorograms of high-copper containing lobules, with zones of maximal copper density corresponding to the zones of the X-fractions detected by ninhydrin.

Comment

The presence of increased amounts of intracellular copper, in spite of the wide difference in the ages (4–40 years) of the subjects studied and the duration of this disease (asymptomatic to 13 years), tends to confirm the impression that the deposition of copper in tissues is a very early feature of hepatolenticular degeneration. Furthermore, it is also evident that this deposition, as far as the liver is concerned, shows an identical pattern irrespective of the type of neurological involvement. Thus, the pseudosclerotic form of hepatolenticular degeneration (Case 1) is quite similar to the juvenile form, with dystonia and rigidity (Cases 2, 3, and 4), in this respect. Nor does the picture differ, except for the advancing cirrhosis, with the duration of clinically overt disease. Thus, patients with a clear, clinically recognizable, disease picture of 13, 8, and 2 years' duration (Cases 1, 2, and 3) show a pattern of copper deposition similar to those in which the signs of disease appeared three months (Case 4) before study or in whom signs were minimal or absent and who were entirely asymptomatic at the time of biopsy (Cases 5 and 7). Although the total amount of copper detected in the latter cases is much less, the main characteristics of the distribution (fine granular deposits, perinuclear plaques) only indicate a difference in the total amount of copper present, rather than a qualitative difference, inasmuch as in those cases where the overt disease was of longer duration (Cases 1, 2, and 3) lobules containing only finely dispersed black granules and others showing the stage of perinuclear plaque formation were interspersed with those lobules showing massive copper deposition as judged by the amount of black copper rubeanate obscuring and filling the whole cell cytoplasm.

The observations on the Cases 4, 5, and 7 are of particular interest in that copper deposition is manifest without any histological evidence of cirrhosis. It appears reasonable to conclude, therefore, that the copper deposition in the liver cell precedes the cirrhotic phase. Thus, the increased affinity for copper demonstrated in our previous study [11] is unlikely to be the result of the cirrhotic process, as is also confirmed by the differences in the copper content of the liver of cirrhotics and patients with hepatolenticular degeneration found at postmortem examination,[3,4,17] established by other workers. It is also noteworthy that the deposition of large amounts of copper with (Cases 1-3) and without (Case 4) evidence of cirrhosis is not accompanied by any evidence of toxic

necrosis of liver tissue, as seen in long-term copper-feeding experiments in animals.[18]

The peculiar appearance of liver-cell nuclei in those lobules not storing copper (Cases 4, 6, 7, 8) appears of significance, especially since the liver biopsy on Case 1, performed in 1947 [15] showed the same picture, although clinical or laboratory evidence of hepatic involvement at the time was very tenuous.

The complete neutrality of the Kupffer cells in terms of participation in the process of deposition of copper in the liver is complementary evidence that the deposition of copper in the liver in hepatolenticular degeneration occurs, not because of an increased "direct-reacting" fraction of copper in plasma but because of a derangement in intracellular metabolism that provides proteins with increased copper-binding affinity. Furthermore, the release of copper from cells undergoing necrosis does not result in phagocytosis of copper proteins by Kupffer cells. At no stage of the disease is the excessively absorbed copper treated as a foreign body (unlike other metals, i. e., Ag, Au, Bi, Mn, Th, etc.), as evidenced by the lack of uptake by Kupffer cells. This is confirmed by the studies of others [13] who failed to find any copper in Kupffer cells in their patients at postmortem examination. This fact, supported by the present study, should find explanation in that intracellular copper released during protein breakdown, or tissue necrosis, is released in a form still bound as a chelate. Perhaps the chelation now is with smaller fragments of peptides instead of the original liver protein, so that this form of copper appears in the tubular fluid and is recovered in the urine as copper oligopeptide complexes.[9,10]

The electrophoretic studies on the soluble liver proteins of Cases 1 and 2 serve to underscore the observation previously made in a surgically removed fresh liver specimen from a patient with only the hepatic form of hepatolenticular degeneration.[11] It is thus clear that the protein fraction that serves to bind the excessive amount of copper is still present and detectable 12 hours postmortem, being indistinguishable in its electrophoretic behavior from that previously studied in specimens removed surgically. The fact that lobules appearing copper-free at the time of examination nevertheless possess the copper-binding proteins also points to these liver lobules as avid reservoirs for additional copper. Thus, the amount of copper which would be removed from the plasma and the rate at which such removal would occur would depend on the number of copper-free lobules still capable of binding copper that the patient under clinical investigation would possess. The greater the proportion of copper-free lobules with respect to total liver mass, the greater would be the uptake and the slower the release of copper. In the opposite instance, when the bulk of the patient's available liver lobules are already saturated with copper, the uptake of additional copper from the plasma would be minimal, while the release of copper from the liver would depend on the rate at which copper-bound liver proteins were catabolyzed. Thus, the results obtained by different workers [6-8,17] who administered Cu^{64} to patients with hepatolenticular degeneration without a knowledge of the state of preexisting copper stores in the liver, cannot be interpreted in terms of defining the disorder in copper metabolism in this disease except insofar as they emphasize that such a disorder does indeed exist.

The present observations also suggest the need for caution in interpreting analytical figures based on the copper content of a liver specimen submitted for microanalysis. Although the increased copper content of the liver found at postmortem examination in long-standing cases of hepatolenticular degeneration is well established,[1-4,13] the need for analytical confirmation of copper enrichment becomes of more than documentary interest in those cases where the diagnosis is doubtful or where the disease is of very short duration. As more and more attention is being directed to the preneurological or the hepatic forms of the disease, it is clear that a diagnosis of hepatolenticular degeneration, especially in the absence of a family

history, would have to depend on biochemical and histochemical evidence, the anatomical evidence of cirrhosis by itself being quite insufficient.

At present, the excessive deposition of copper in tissues, especially the liver, appears to be the most reliable criterion for the establishment of a diagnosis even in the earliest and hepatic forms of the disease.[17] For these reasons, although the finding of a fivefold to tenfold increase in the copper content (for fresh or dry weight) of the liver by quantitative analysis should be sufficient evidence for the existence of the biochemical stigma of the disease, the absence of such an elevation or only a small but significant increase requires further investigation. The fact that great unevenness in the copper content of individual lobules does exist in this disease, so that one copper-laden lobule can be surrounded by dozens of lobules without any copper in them whatsoever, indicates the main source of possible error in conclusions drawn from the analysis of tissue alone. The error could be eliminated if a very large specimen (50-100 gm. tissue) were compared with a similar-sized normal liver. But this is not usually done because of the technical impracticability. Thus, the most careful chemical quantitation would only be a gross approximation of the total copper content of a liver. These handicaps become all the more pronounced when the accumulation of copper is minimal, in the early, or presymptomatic, stage of the disease. It is in these cases that the histochemical localization of copper becomes of paramount importance, not only in establishing an unequivocal diagnosis, but also in tracing the role of copper deposition in the pathogenesis and natural course of the disease. The extreme usefulness of histochemical localization of copper in diagnosis of the hepatic form of the disease by liver needle biopsy has already been pointed out elsewhere.[17] The experience reported in this study also indicates that liver biopsy can be extremely useful in the earliest, or asymptomatic, stages of the disease.

Attention should also be directed to the clinical features of the cases presented in this study. The unremitting course of the cirrhosis of the liver in spite of satisfactory control of nervous signs and symptoms by dimercaprol therapy is illustrated by Case 1. Case 3 and the pertinent family history, as well as the family represented by Cases 4-8, attest to the fact that a great number of asymptomatic members of a family can be affected with the stigmata of the disease, as pointed out previously.[9] Thus, there is nothing "atypical" in this feature, as has been claimed by some.[7,4] The fact that siblings of a collateral branch were similarly affected (Cases 4 and following) is extremely unusual and not only points to our incomplete understanding of the transmission of hepatolenticular degeneration by asymptomatic genetic carriers but also underlines the danger of considering asymptomatic siblings as "normal" on the basis of only an isolated biochemical test performed on blood alone.

To students of the Kayser-Fleischer ring, Cases 4, 5, and 7 present information of a very unusual nature. Although the corneal rings were quite well developed in these siblings, the accumulation of copper in the tissues, as judged by the liver biopsy, was only pronounced in one (Case 4), while the other two siblings had very minimal amounts of increase in hepatic intracellular copper. This finding agrees with our conclusions on the nature of the Kayser-Fleischer ring based on histochemical and electron-microscope studies.[20] The deposition of copper in the corneal ring in a characteristic pattern, as described, occurs secondarily to a preexisting anomalous matrix in Descemet's membrane, due to the primary protein disorder of hepatolenticular degeneration, and thus is not a result of supersaturation of tissue fluids with ionic copper. The material presented here, therefore, confirms this view in that well-developed Kayser-Fleischer rings can be manifest in the presence of only minimally increased copper deposition in tissues that have a very high affinity for copper in this disease.

Summary

Liver tissue obtained from two patients with hepatolenticular degeneration at autopsy and from six patients by biopsy was studied with respect to the intrahepatic distribution of copper, with use of an improved modification of the rubeanic acid histochemical method.

There was a marked variation in the copper content of individual lobules in each patient, so that lobules filled with copper-laden cells were surrounded by copper-free lobules. The intracellular distribution of copper presented characteristic stages consisting of (1) fine diffuse distribution of copper throughout the liver-cell cytoplasm, (2) perinuclear "caps" and plaques, and (3) coarse heavy deposits throughout liver-cell cytoplasm. These appeared to represent progressive stages of increasing amounts of copper deposited. The distribution of copper was similar in the pseudosclerotic and juvenile forms of the disease. Kupffer cells were uniformly free of copper.

Copper deposition was shown to be an early feature of the disease, preceding the cirrhosis. The amount of copper deposited bore no relationship to whether the patients were symptomatic or not.

By means of paper electrophoretic studies it was shown that those liver lobules that appeared to be copper-free were nevertheless potential sites for further copper deposition by virtue of their content of abnormal protein constituents.

The pathogenesis of the disease is discussed in the light of these findings.

Dr. D. Denny-Brown suggested the study of the intrahepatic distribution of copper for possible differences in the copper contents of older and regenerating lobules and gave advice in the course of this investigation.

REFERENCES

1. Haurowitz, F.: Über eine Anomalie des Kupferstoffwechsels, Ztschr. physiol. Chem. 190:72-74, 1930.

2. Mandelbrote, B. M.; Stanier, M. W.; Thompson, R. H. S., and Thruston, M. N.: Studies on Copper Metabolism in Demyelinating Diseases of the Central Nervous System, Brain 71:212-228, 1948.

3. Cumings, J. N.: Copper and Iron Content of Brain and Liver in the Normal and in Hepato-Lenticular Degeneration, Brain 71:410-415, 1948.

4. Cartwright, G. E.; Hodges, R. E.; Gubler, C. J.; Mahoney, J. P.; Daum, K.; Wintrobe, M., and Bean, W. B.: Studies on Copper Metabolism: XIII. Hepatolenticular Degeneration, J. Clin. Invest. 33:1487-1501, 1954.

5. Uzman, L. L.: Histochemical Localization of Copper with Rubeanic Acid, Lab. Invest. 5:299-305, 1956.

6. Bearn, A. G., and Kunkel, H. G.: Localization of Cu^{64} in Serum Fractions Following Oral Administration: An Alteration in Wilson's Disease, Proc. Soc. Exper. Biol. & Med. 85:44-48, 1954.

7. Bearn, A. G., and Kunkel, H. G.: Abnormalities of Copper Metabolism in Wilson's Disease and Their Relationship to the Aminoaciduria, J. Clin. Invest. 33:400-409, 1954.

8. Matthews, W. B.: The Absorption and Excretion of Radiocopper in Hepato-Lenticular Degeneration (Wilson's Disease), J. Neurol. Neurosurg. & Psychiat. 17:242-246, 1954.

9. Uzman, L. L., and Hood, B.: The Familial Nature of the Amino-Aciduria of Wilson's Disease (Hepatolenticular Degeneration), Am. J. M. Sc. 223:392-400, 1952.

10. Uzman, L. L.: On the Relationship of Urinary Copper Excretion to the Aminoaciduria in Wilson's Disease (Hepatolenticular Degeneration), Am. J. M. Sc. 226:645-652, 1953.

11. Uzman, L. L.; Iber, F. L.; Chalmers, T. C., and Knowlton, M.: The Mechanism of Copper Deposition in the Liver in Hepatolenticular Degeneration (Wilson's Disease), Am. J. M. Sc. 231:511-518, 1956.

12. Zimdahl, W. T.; Hyman, I., and Cook, E. D.: Metabolism of Copper in Hepatolenticular Degeneration, Neurology 3:569-576, 1953.

13. Okinaka, S.; Yoshikawa, M.; Toyoda, M.; Mozai, T.; Toyokura, Y., and Kameyama, M.: Pathogenesis of Hepatocerebral Disease: II. Histochemical Study of Copper of Liver and Brain in Wilson's Disease, A. M. A. Arch. Neurol. & Psychiat. 72:573-578, 1954.

14. Green, C. L.: Histochemical Demonstration of Copper in a Case of Hepatolenticular Degeneration, Am. J. Path. 31:545-553, 1955.

15. Uzman, L., and Denny-Brown, D.: Amino-Aciduria in Hepatolenticular Degeneration (Wilson's Disease), Am. J. M. Sc. 215:599-611, 1948.

16. Denny-Brown, D., and Porter, H.: The Effect of BAL (2,3-Dimercaptopropanol) on Hepatolenticular Degeneration (Wilson's Disease), New England J. Med. 245:917-925, 1951.

17. Chalmers, T. C.; Iber, F. L., and Uzman, L. L.: Hepatolenticular Degeneration (Wilson's Disease) as a Form of Idiopathic Cirrhosis, New England J. Med. 256:235-242, 1957.

18. Gubler, C. J.; Taylor, D. S.; Eichwald, E. J.; Cartwright, G. E., and Wintrobe, M. M.: Copper Metabolism: XII. Influence of Manganese on Metabolism of Copper, Proc. Soc. Exper. Biol. & Med. 86:223-227, 1954.

19. Earl, C. J.; Moulton, M. J., and Selverstone, B.; Metabolism of Copper in Wilson's Disease and in Normal Subjects: Studies with Cu-64, Am. J. Med. 17:205-213, 1954.

20. Uzman, L. L., and Jakus, M. A.: The Kayser-Fleischer Ring: A Histochemical and Electron Microscope Study, Neurology, 7:341-355, 1957.

News and Comment

PERSONAL

Dr. Stanley P. Reimann.—Dr. Stanley P. Reimann, of Philadelphia, has been awarded the Strittmatter gold medal of the Philadelphia County Medical Society in recognition of his many years of productive work in the cancer field.

Dr. William E. Jaques Takes Position at University of Oklahoma.—Dr. William E. Jaques, Associate Professor in the Department of Pathology at the Louisiana State University School of Medicine, on June 1, 1957, assumed the chairmanship of the Department of Pathology at the University of Oklahoma School of Medicine.

Dr. Ernest W. Goodpasture Receives Honorary Degree.—Dr. Ernest W. Goodpasture, Scientific Director of the Department of Pathology at the Armed Forces Institute of Pathology, was awarded the honorary degree of Doctor of Laws on June 3, 1957, at the commencement exercises at Tulane University School of Medicine, New Orleans. Dr. Goodpasture addressed the members of the graduating class of the School of Medicine on the subject of "Medicine and the Ivied Walls."

Dr. Virgil H. Moon Leaves University of Miami.—Dr. Virgil H. Moon has resigned his position as Research Professor of Pathology at the University of Miami School of Medicine. Dr. Moon has been at the University of Miami since his retirement as Professor of Pathology at Jefferson Medical College of Philadelphia, in 1948.

Appointment for Dr. Hugh G. Grady.—Dr. Hugh G. Grady has been appointed Professor of Pathology and Director of the Department at the Seton Hall School of Medicine. He left his duties at the Armed Forces Institute of Pathology on June 1.

Award for Dr. Jonas Salk.—Dr. Jonas Salk received the 1957 Howard Taylor Ricketts Award of the University of Chicago on May 17. He spoke on the subject "Poliomyelitis from the Perspective of Biology."

ANNOUNCEMENTS

Postgraduate Course in Forensic Pathology.—A postgraduate course in forensic pathology is to be given at the Armed Forces Institute of Pathology, Washington, D. C., from Nov. 4 through Nov. 8, 1957. Further information can be obtained from the A. F. I. P., Washington 25, D. C.

Training Seminar in Diagnostic Use of Radioisotopes.—A training seminar for pathologists in the diagnostic use of radioisotopes, sponsored by the American Society of Clinical Pathology and the Oak Ridge Institute of Nuclear Studies, is to be held at the Medical Division, Oak Ridge, Tenn.

The seminar has been set up specifically for certified pathologists and designed to meet the recommendations and requirements of the Atomic Energy Commission. This seminar will furnish a reasonable introduction to radioisotopes for those pathologists who are principally

interested in diagnosis but who may be called on to assist and advise on therapeutic uses of radioisotopes. The program will consist of two one-week meetings separated by a three-month interval. The first (basic) week will consist mainly of fundamental techniques and concepts. The second (clinical) week will be held three months after the first and will be devoted mainly to the practical application of these principles. It is expected that in the intervening three months the participating pathologists will visit laboratories in their home areas which are already approved for the use of isotopes and will assimilate relevant literature. At the end of the second week the Medical Division of ORINS will give to participants a statement of preceptorship in the diagnostic uses of radioisotopes. It is emphasized that while this does not automatically qualify any person for AEC permission to use isotopes, the course is designed to meet the training requirements of the Isotopes Extension of the AEC.

The training seminar will consist of approximately 50% lectures and demonstrations and 50% laboratory work. Each participant will be expected to administer to himself a dose of less than 10μc. of I^{131} to accomplish a thyroid uptake or a blood volume measurement.

The teaching staff has been selected from pathologists, internists, surgeons, radiologists, physicists, and biochemists in and around Oak Ridge or members of the Council on Radioisotopes, ASCP. All are experienced in the clinical use of radioisotopes.

The two one-week programs are considered as a unit, and application must be made for both. The first week will be held Nov. 11-16, 1957; the second week, Feb. 10-15, 1958.

Requirements: Applicants must be certified in either clinical pathology or pathologic anatomy or be members or fellows of the American Society of Clinical Pathology, must be United States citizens, and must have a license to practice medicine. Applications will be reviewed by the Council on Radioisotopes, ASCP. Qualified candidates will be accepted in chronologic order.

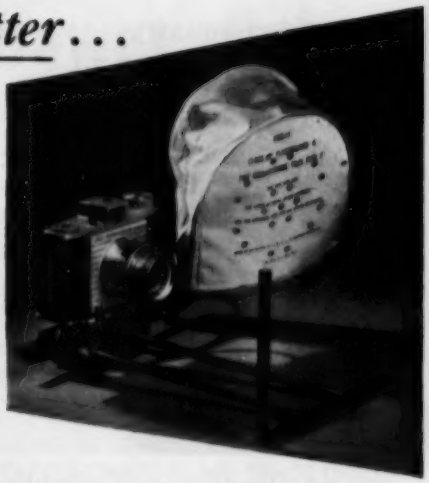
Housing: For assistance in obtaining housing, write to Dr. Harold Steffee, Oak Ridge Institute of Nuclear Studies, P. O. Box 117, Oak Ridge, Tenn.

Fee: Entire course, $25, payable to the Oak Ridge Institute of Nuclear Studies on the first Monday.

Application: Application for this training seminar should be made to Dr. Oscar B. Hunter Jr., Chairman, Council on Radioisotopes, American Society of Clinical Pathology, Suite 1000, Columbia Medical Building Annex, 915-19th St., N. W., Washington 6, D. C. The first training seminar will be limited to 16 pathologists.

To help you say it better...

Here are 2 "musts"...



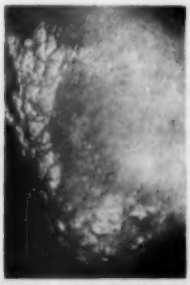
1. The Kodak Technical Close-Up Outfit

... records what you see *when you see it*

It's a complete outfit with Kodak Pony 828 Camera, Kodak B-C Flasholder, stainless steel bracket and field frame, Kodak Close-Up Flashguards A and B, and a Kodak Portra Lens with fittings.

About all you have to do is load camera with film, insert flash bulb, cock shutter and—shoot. List, $62.50.

Prices include Federal Tax and are subject to change without notice.



Close-ups: Hold field frame at subject. Area covered—3⅛ x 4⅛ in.

Half-length or regional pictures: Hold camera at 2½ feet from the subject.

Full-length pictures: Hold as viewfinder indicates—usually 7 to 8 feet from the subject.

2. The new Kodak 300 Projector

... shows what you saw *whenever you wish*

Big-projector brilliance without bulk or bother. Gives you extra-bright, uniform screen illumination for 2 x 2 slides. Focusing and elevation controls on top, right at your finger tips.

With new Kodak Readymatic Slide Changer holds up to 36 2 x 2 slides, $64.50 list.

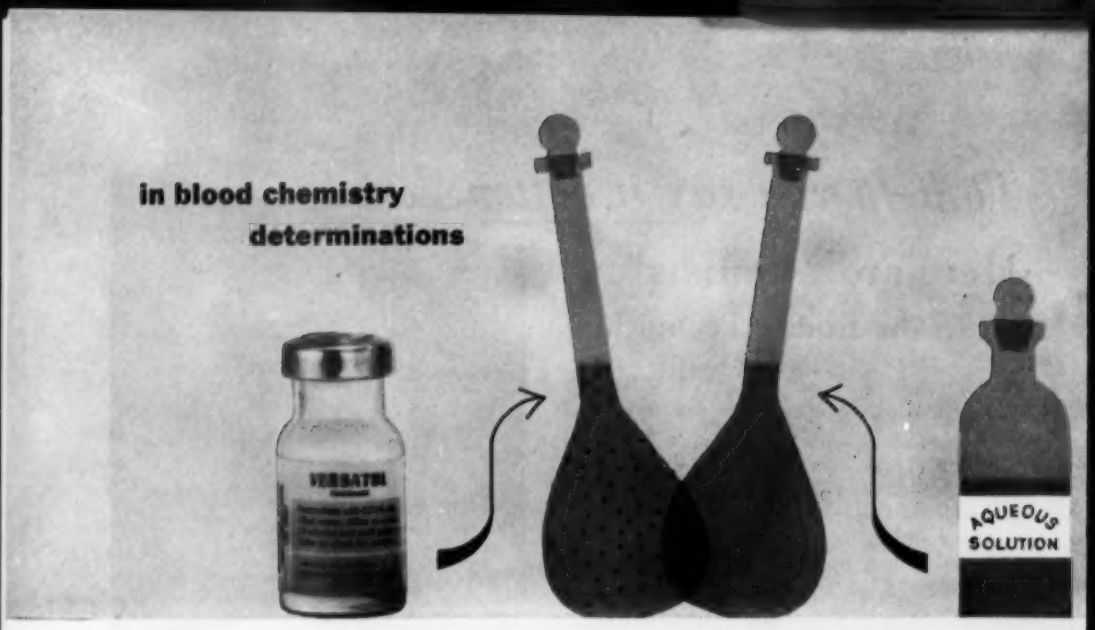
With Automatic Magazine Changer stores and shows up to 36 2 x 2 slides, $74.50 list.

For further details, see your Kodak photographic dealer or write:

EASTMAN KODAK COMPANY
Medical Division, Rochester 4, N. Y.

Kodak

Serving medical progress through Photography and Radiography



you can eliminate protein error...

Examine the two flasks above: one represents a highly complex, protein-containing solution — serum; the other, a standard containing a single substance in distilled water. Comparing two such completely different solutions can lead to protein error. For example, in determining urea nitrogen by direct nesslerization, urease-treated Versatol filtrates and blood filtrates develop color at the same rate (maximum color in 30 minutes). Aqueous urea standards develop maximum color in 14 minutes. Obviously, comparing aqueous standards with blood filtrates leads to protein error.

Duplicates Patient's Blood. Versatol, the biologically true, biologically ideal blood chemistry standard containing virtually all serum proteins, eliminates or cancels out protein error. It replaces aqueous standards in calibrating instruments, titrating unknowns and in flame photometry. Each determination is performed in the presence of the same interfering substances, thus avoiding differences leading to inaccuracies. For example, the protein component of serum combines readily with dyes, changing their color characteristics. Also, the portion of calcium bound to protein cannot be measured against an aqueous standard.

Standard AND Control. Versatol can also be used as a *control* to check equipment, reagents and technique. It contains known normal amounts of the following 12 constituents: serum protein-bound iodine, total nitrogen, total protein, nonprotein nitrogen, urea nitrogen, creatinine, sodium, potassium, chloride, phosphate, calcium and glucose.

Versatol is ready for use with just the addition of distilled water. Available from your local laboratory supply distributor in boxes of three 5 ml. vials, $7.50, and ten 5 ml. vials, $21.00.

Versatol
TRADEMARK

Laboratory Supply Division
WARNER-CHILCOTT
MORRIS PLAINS, N. J.

V

Although not yet using Versatol, I've heard much about it from my colleagues.

Please send me

- ☐ More information on Versatol
- ☐ Part II of Warner-Chilcott's Blood Chemistry Manual

Please ship ..... boxes of Versatol 10's or ..... boxes of Versatol 3's through my nearest distributor.

NAME _____

LABORATORY _____

ADDRESS _____

CITY _____ STATE _____

Paragon Tray Drawer Cabinet

Compact

Low Cost

FOR FILING
MICROSCOPIC SLIDES 3 x 1″
KODACHROME TRANSPARENCIES
2 x 2″ SLIDES
LANTERN SLIDES
(up to 3¼ x 4¼)
PETROGRAPHIC SLIDES

When you purchase a
PARAGON TRAY DRAWER CABINET
YOU PURCHASE FILING SPACE ONLY
NO WASTE SPACE-EVERY INCH USED

U. S. Pat. No. 2,202,047
C101—Tray Drawer Cabinet for 3 x 1 Micro Slides
Capacity 4500—18¾ x 15¾ x 4¼

All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimensions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.

C221—Capacity 1500 Slides—18¾ x 11 x 3¼
For Filing KODACHROME TRANSPARENCIES and 2 x 2″ SLIDES

SPECIFICATIONS: All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

Address your orders and inquiries to Dept. P.

Manufactured Exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N.Y.